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Support Document Test Data Development Standards, Chronic Health Effects Toxic Substances Control Act, Section 4

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Washington, D. C.

May 1979

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Support Document Test Data Development Standards:

Chronic Health Effects

Toxic Substances Control Act Section 4

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The Proposed Chronic Toxicity Test Standards (F.R.), written under Section 4(a) and 4(b) of the Toxic Substances Control Act (TSCA), are designed to ensure the development of reliable and adequate test data for assessing the chronic health effects of natural and synthetic chemicals. Moreover, the testing requirements are not to create unnecessary economic barriers to technological innovation in the chemical industry. To fulfill these responsibilities, scientists and staff of the Environmental Protection Agency (EPA) have reviewed the literature and discussed the significant scientific and economic issues both in Agency Workgroup meetings and with consultants and reviewers from outside the Agency. The results of this effort are reflected in detail in this Support Document and the Preamble to the Chronic Health Effects Standards.

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SUPPORT DOCUMENT
TEST DATA DEVELOPMENT STANDARDS:
CHRONIC HEALTH EFFECTS

TOXIC SUBSTANCES CONTROL ACT
SECTION 4

MAY 1979

HEALTH REVIEW DIVISION
OFFICE OF TOXIC SUBSTANCES
ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

PREFACE

The Proposed Chronic Toxicity Test Standards (F.R.), written under Section 4(a) and 4(b) of the Toxic Substances Control Act (TSCA), are designed to ensure the development of reliable and adequate test data for assessing the chronic health effects of natural and synthetic chemicals. Moreover, the testing requirements are not to create unnecessary economic barriers to technological innovation in the chemical industry. To fulfill these responsibilities, scientists and staff of the Environmental Protection Agency (EPA) have reviewed the literature and discussed the significant scientific and economic issues both in Agency Workgroup meetings and with consultants and reviewers from outside the Agency. The results of this effort are reflected in detail in this Support Document and the Preamble to the Chronic Health Effects Standards.

The Support Document is not intended to be a comprehensive scientific treatise which reviews all the literature pertinent to chronic toxicity testing. However, the review and dialogue with scientists were thorough, wide-ranging, and thoughtfully performed in order that reasonable and effective standards could be developed. Along with the Preamble, the Support Document identifies and discusses in detail the most significant issues that pertain to the proposed standards and records the Agency's reasoning behind their development.

The EPA encourages all interested parties to review the scientific and economic reasoning expressed in the Support Document and provide comment to the Agency. Such input can significantly benefit the development of Final Test Standards. All comments will be carefully reviewed by EPA, and all major points will be addressed in the final Support Document.

Written comments should bear the document control number EPA 560/11-79-001 and should be submitted to the Document Control Officer (TS-793), Office of Toxic Substances, U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, D.C. 20460.

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Support Document
Test Data Development Standards:
Chronic Health Effects
Toxic Substances Control Act
Section 4

Introduction

Under Sections 4 (a),(b) of the Toxic Substances Control Act (TSCA), the Administrator of the Environmental Protection Agency (EPA) is proposing standards for testing chemicals for oncogenic and non-oncogenic chronic effects induced in laboratory animals after long-term, repeated or continuous exposure. Health effects which are irreversible, of long duration, or occur only after long-term exposure are classified as chronic[1]. The objectives of Chronic Tests are to detect such toxic effects in all affected target organs and systems and define toxic and no-observed-effect levels (NOEL) for Non-oncogenic chronic effects in order to develop data for assessing human risk.

Many chemical agents in the environment are capable of interacting with biological systems and, thus, of inducing chronic health effects (NAS, 1975). The irreversible or long-continuing nature of these health effects and the insiduous way they may develop--perhaps years after a single exposure or the initiation of long-term, low-level exposure to a toxic chemical substance--make testing of chemicals for chronic toxicity a necessary regulatory measure for disease prevention (Hayes, 1975; NAS, 1975; NAS-NRC, 1977).

So that the public might understand the scientific and economic rationale behind the development of the Chronic Health Effects Test Standards, the Administrator of EPA is making available the Support Document for these Standards. The document addresses issues relevant to the design and conduct of chronic toxicity tests, discusses the costs of these tests and, in addition, provides other background information pertinent to understanding the need for and the implementation of

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[1] For a discussion of EPA's use of the term oncogenicity and other cancer-related terms, consult "Definitions," Appendix A to Section I of this document.

Chronic Health Effects Test Standards. The Support Document is composed of the following sections:

I. Need for Chronic Toxicity Studies

II. Scientific Aspects of the Proposed Standards

III. Economic Issues

IV. Confidentiality Issues

V. Differences Between TSCA Test Standards and FIFRA Guidelines

I. Need for Chronic Toxicity Studies

- A. Environmental Factors and Chronic Effects
- B. Environmental Chemicals and Chronic Health Effects: The Need for Chronic Toxicity Testing
- C. Impact of Chronic Disease on Health in the United States
- D. Economic Costs of Chronic Disease
- E. Environmental Factors Associated with Oncogenicity in Man
    - 1. Physical Factors
    - 2. Biological Factors
    - 3. Chemical Factors
- F. Initiation, Promotion, and Other Chemical Interactions Which Enhance Oncogenic Potential of Low-Level Chemical Exposure
    - 1. Cocarcinogenesis
        - a. Initiation and Promotion
        - b. Additive and Synergistic Interactions
    - 2. Metabolic Activation of Chemicals and Carcinogenicity
- G. Identification of Chemicals with Chronic Health Effects: The Use of Epidemiology, Short-Term Tests for Predicting Oncogenicity, and Long-Term Chronic Toxicity Tests
    - 1. Epidemiology
    - 2. Short-Term Tests for Predicting Oncogenicity
    - 3. Long-Term Chronic Toxicity Tests
        - a. Oncogenicity Tests
        - b. Non-oncogenicity Chronic Toxicity Tests

Definitions: Appendix A

References

Tables and Figures

I. Need for Chronic Toxicity Studies

A. Environmental Factors and Chronic Health Effects

In its report Human Health and the Environment: Some Research Needs (1977), the Second Task Force for Research Planning in Environmental Health Science characterized the relationship between environmental factors and chronic health effects in the following way:

> There is virtually no currently-recognized chronic disease to which environmental factors are not contributing, either (a) by direct or indirect action on the organ itself, or (b) through an influence on modulating mechanisms of neuroendocrine or immunologic nature. For everyone of the multitude of cardiovascular, pulmonary, renal, hepatic, pancreatic, gastrointestinal, cutaneous, and neurologic diseases as well as disorders of the hematopoietic system, examples exist of toxic manifestations brought about by direct actions of environmental stresses.

Environmental factors, such as chemicals, radiation, infectious agents, drugs, smoking, nutritional deficiency and other factors related to diet, have been associated with chronic health effects (T.F., 1978; S.T.F. , 1977). The effects may range, for example, from a subclinical, sustained impairment of the respiratory tract evidenced only under stress, to chronic diseases such as emphysema, arteriosclerosis, diabetes mellitus, cirrhosis of the liver, nephrosis of the kidney, and benign or malignant tumors, to premature death as a delayed response to a toxic substance gradually accumulated in the body (Albert, 1975; Blodgett et al. 1975). However, the data base for evaluating the relationships between most environmental factors and chronic health effects is presently inadequate or not available to government scientists. Recognizing this problem, Congress enacted the Toxic Substances Control Act to ensure the availability or development of adequate test data for regulatory decision-making with regard to chemical substances and mixtures.

B. Environmental Chemicals and Chronic Health Effects: The Need for Chronic Toxicity Testing

The large and diverse productivity of the chemical industry and the wide-spread use of chemicals in modern industrial and agricultural societies ensure that humans will continue to undergo long-term exposure to exogenous chemicals. Chemicals in commerce around the world may number as high as 100,000 with another 1000 new chemicals coming on the market each year. About 63,000 chemicals are currently in common use (Maugh, 1978).

In the United States alone, approximately 45,000 chemicals are in current production, according to the chemical inventory under development by EPA. In 1973 the production of the top fifty chemical substances in the United States was about 410 billion pounds (C&EN May 6, 1974). In 1977, production of organic chemicals indicated on C&EN's "Top 50 Chemical Products List" was 156.9 billion pounds, an 8 percent increase over 1976. The production for the 50 largest-volume chemicals in 1977 was 482.7 billion pounds -- a production record -- and indicators suggest that for 1978, production may reach 500 billion pounds (C&EN May 1, 1978). These figures do not include the production of inorganic or organic chemicals produced in lesser volumes.

Some of these chemicals which enter the environment undoubtedly are capable of inducing chronic health effects in man. These effects may take years to manifest themselves, however, and establishment of causal relationships will be very difficult. Consequently, suspect chemical must be identified and carefully laboratory tested.

Approximately 6000 chemicals have been laboratory-tested for toxicity--about 6 percent of the chemicals in commerce. The quality and uniformity of the testing, however, varies significantly. EPA's study Preliminary Assessment of Suspected Carcinogens in Drinking Water, (1975) demonstrates the need for a regulatory testing program for chronic toxicity of environmental chemicals. Of the 253 organic chemicals identified in drinking water, a large number lack sufficient data for evaluating whether they produce tumors, gene mutations, birth defects or other serious chronic effects. Among those organic chemicals identified were chloroform, carbon tetrachloride, benzene, haloethers, chloro-olefins, and polynuclear aromatic hydrocarbons--all of which have been associated with carcinogenesis either in man or laboratory animals. Preliminary epidemiologic studies of aggregate populations in Louisiana, Ohio, and New Jersey support the hypothesis that carcinogens in drinking water are related to the occurrence of human cancer (Harris, Page and Reiches, 1977).

Since chemicals in surface and drinking water are prime contenders for entering the human food chain at low dose levels and repeated exposure, the lack of knowledge concerning their chronic toxicity is particularly disturbing and, moreover, argues for an effective chronic toxicity testing program for chemicals.

The Task Force on Environmental Cancer and Heart and Lung Disease (1978), composed of scientists from EPA, NCI, NHLBI, NIOSH, and NIEHS, has thus far focused its attention on chemical pollutants and found evidence associating them with cancer and heart and lung disease, but the evidence is "diffuse and in many cases inconclusive." Known or suspected relationships which the Task Force identified are in Figure 1.

As Figure 1 indicates, of the three diseases, most is known about the association between environmental chemicals and cancer. As of December 1977, the International Agency for Research on Cancer (IARC) had evaluated 368 chemicals selected according to 2 main criteria:

a. that there is evidence of a human exposure;

b. that there is some evidence of carcinogenicity in experimental animals and/or some evidence or suspicion of human risk.

IARC reported that 26 chemicals or industrial processes were associated epidemiologically with cancer in humans (Table 1), and for 221 chemicals (Table 2), some "evidence of carcinogenicity was found in at least one species of experimental animals" (Tomatis et al., 1978). The remaining 121 chemicals lacked sufficient data for an adequate evaluation; however, most likely some of these as well as additional synthetic and natural chemicals will eventually be associated with human cancer.

Evidence linking environmental chemicals with non-oncogenic diseases is not as well-developed as for cancer; however, there are clear indicators that chemically-induced non-oncogenic health effects pose a considerable health problem. The causal role of asbestos in the chronic human disease asbestosis is well known (Merewether, 1930). The association between 'soft' water and the death rate from cardiovascular disease has been verified by studies in Japan, United States, United Kingdom, and Sweden although the chemical causal factors have not yet been clearly identified (Kobayashi, 1957; Schroeder, 1974). Research such as Schroeder's study of recondite toxicity of trace elements in laboratory animals (1973) -- a study of "subtle metabolic changes consistent with reasonable survival" associated with trace elements -- indicates possible association between industrial elements and chronic diseases. Signs of recondite toxicity include hypertension, atherosclerosis, diabetes mellitus, coronary artery occlusion, benign and malignant tumors, as well as alterations in carbohydrate and lipid metabolism, glycosuria, proteinuria, shortened longevity, reproductive abnormalities and weight loss in older animals.

Other studies in both humans and laboratory animals have shown association between environmental chemicals and the expression of chronic diseases. For example, a relation has been shown between long-term occupational exposure to vinyl chloride and the oncogenic effect, angiosarcoma of the liver, and also the non-oncogenic effect, chronic liver injury--a rather nonspecific periportal fibrosis which in advanced stages resembles primary biliary cirrhosis (Thomas et al., 1975). There have been associations between long-term exposure to fluorocarbons and cardiac palpitation in the human (Speizer et al., 1975), and long-term exposure to hydrocarbons and rapidly progressing glomerulonephritis in the human kidney (Beirne and Brennan, 1972; Harman, 1971; Zimmerman et al., 1975). Moreover, experimental toxicology has demonstrated that B-cell destruction or dysfunction in the pancreas can be caused by chemicals, suggesting that some diabetes might result from exposure to toxic chemicals in the environment (Longnecker, 1977).

These findings attest to the diversity of adverse chronic health effects associated with chemical exposure. But typically, the chronic effects are subtle and difficult to study directly. Exposures in the real world are usually small; their effects may often be attributed to other agents to which the subject is exposed voluntarily as well as involuntarily. However, the biological implications of long-term, low-level exposure "may be quite serious--especially for chronic degenerative disease" (Burger, 1976). Therefore, the need for testing selected chemicals for these effects is critical.

C. Impact of Chronic Disease on Health in the United States

The occurrence of chronic disease has an extraordinarily large impact on health in the United States. The precise proportion of the impact directly or indirectly related to chemicals in the environment is unknown. NIOSH has estimated that as many as 100,000 deaths each year result from diseases induced by or related to occupational exposures to toxic substances in the workplace. In addition, 390,000 new cases of occupationally related diseases are estimated to be occurring annually (Ashford, 1976). Numerous studies have demonstrated a higher incidence of particular chronic conditions in human populations exposed to certain chemicals in the workplace. The same chronic diseases occurring in the general population may also be related to chemical exposure.

The National Center for Health Statistics commissioned the U.S. National Committee on Vital and Health Statistics to assess this problem and make recommendations concerning the collection of statistics required to determine the health effects of a variety of environmental agents (NCHS, 1977). Until such data are available, however, the impact of chemicals on chronic illness in the United States can only be suggested by considering the overall impact of chronic disease on the U.S. population and assuming a certain percentage is associated with environmental chemicals.

In 1975, mortality rates (per 100,000 population) for chronic diseases (Vital Statistics of the U.S., 1975) were as follows: diseases of the heart (leading cause of death in the U.S.) 336.2; cancer, 171.7; diabetes mellitus, 16.5; cirrhosis of the liver, 14.8; arteriosclerosis, 13.6; and emphysema, 8.8.

Of the 2 million people who died in 1975, 1,028,415 succumbed to diseases of the circulatory system, 365,538 to neoplasms, 109,276 to disease of the respiratory system, 73,189 to disease of the digestive system, and 28,029 to disease of the genitourinary system (Paringer and Berk, 1977).

Prevalence of non-oncogenic chronic conditions as estimated by the National Center for Health Statistics totals approximately 174.1 million cases: chronic circulatory conditions 36.4 million; (Wilder, 1974) chronic skin and musculoskeletal conditions 25.2 and 25.4 million respectively (Wilder, 1974); chronic conditions of the genitourinary, nervous, endocrine, metabolism, blood and blood-forming systems 23.2 million (Scott, 1977); selected chronic respiratory conditions 46.8 million (Wilder, 1973); and selected chronic digestive conditions, 17.0 million (Wilson, 1973).

With regard to cancer, of the present population in the United States, nearly 55 million people (one out of four) will eventually develop the disease; approximately 700,000 new cancer cases are expected in 1978, an increase from 675,000 in 1976 and 690,000 in 1977 (Cancer Facts and Figures, 1978). Furthermore, since cancer usually results in death if it is not treated successfully, less than half of all cancer patients live five years after first diagnosis (Cancer Facts and Figures, 1978; Cairns, 1975). The American Cancer Society estimates that 390,000 people will die from cancer in 1978.

Furthermore, the ability of a large number of people to pursue their lives actively is significantly affected by chronic disease. According to the National Center for Health Statistics, "In 1974, almost 7 million people or 3.3 percent of the noninstitutionalized population were unable to perform what they considered their major activity, 7.3 percent were limited in the kind or amount of major activity, and 3.5 percent were limited in other activities as a direct result of chronic diseases." In total, about 30 million persons had some degree of limitation of activity as a result of chronic diseases (Wilder, 1977).

D. Economic Cost of Chronic Disease

The cost of health care for people who suffer from chronic disease is very large. For example, (using a 4 percent discount for lost future earnings) in fiscal year 1975, the total economic cost for diseases of the circulatory systems was approximately $50.4 billion, for diseases of the respiratory system $19.7 billion, for diseases of the digestive system $22.8 billion and disease of the genitourinary system $8.2 billion (Berk, Paringer and Mushkin, 1978).

The economic burden which cancer alone inflicts on society is extraordinarily large. The General Accounting Office of the United States (GAO) estimated in 1976 the cost of cancer to be $15 billion per year, 3.5 billion of which goes to care and treatment and the remainder to loss of earning power and productivity (GAO, 1976).

Rice and Hodgson (1978) of the National Center for Health Statistics recently reported, however, the total economic cost of neoplasms in 1975 was in the range of $19 to $22 billion which breaks down into direct cost of $5.3 billion and indirect costs ranging from $13.6 to $17.0 billion, depending on the discount rate (Cf. Paringer and Berk, 1977). Direct costs are outlays for prevention, detection, and treatment for the illness and indirect costs are the loss in the economy's output because of disability, morbidity and premature death of workers (NCHS, 1977).

If "social costs" are computed into the estimate, the yearly cost of cancer soars even higher. Abt (1975) attempted to quantify social costs of cancer--costs of psychosocial deteriorations that are brought about by disease but are not reflected in the direct and indirect economic costs--and estimated the minimum annual social cost of cancer "to be about $2.5 billion, excluding extinction costs and about $138 billion if costs of extinction are included." (Extinction costs are the collective social costs of cancer mortality.) Consequently, the annual financial burden of cancer on people and society in the United States may be as high as $150 billion.

Moreover, between 1950 and 1976, total health expenditures rose at an average rate of 9.9 percent, and in 1976 expenditures were 14 percent greater than in the previous year. Monetary costs for health care will continue to rise, suggesting that the economic burden of chronic disease upon society is ever increasing.

Furthermore, the average age of the population has grown older, a trend, it is thought, that will continue (NCHS, 1977):

> Assuming that women average 2.1 births and that recent death rates prevail, the total population will be about 262.5 million in the year 2000. The number of children under age 20 will increase by only 6.3 percent (from 74.6 million to 79.3), while the number of elderly people will increase by 36.6 percent (from 22.4 million to 30.6 million).

Given this aging of the population, the incidence of chronic diseases and, therefore, treatment-related costs to society are expected to increase since these diseases are found most commonly in the middle and elderly age groups.

If the incidence of chronic disease could be reduced by prevention--by testing chemical substances for chronic health effects--even if the reduction is only a small fraction, economic and health gains to society would be significant. The "Forward" to the study of environmental health issues, Human Health and the Environment: Some Research Needs (1977), states:

> This report is produced against a background of our nation's increasing recognition that:
>
> 1. The costs of medical diagnosis and treatment are astronomical and growing; a further increase in investments in these areas will not yield significant improvements in average longevity, productivity, or quality of life;
> 2. Prevention of illness, disability, and premature death will yield the greatest benefits to society; and
> 3. Identification, evaluation, and subsequent modification of the role of environmental factors in causing illness and premature death promise early and major payoffs in the prevention and control of disease.

The TSCA reflects this view; testing of selected chemicals is an effective way of modifying the role of environmental factors in causing illness and premature death.

E. Environmental Factors Associated with Oncogenicity in Man

The percent of cancers among the human population in the United States caused by environmental factors has been variously estimated from 70 to 90 percent (Higginson and Muir, 1973). These percentages are essentially based on epidemiological studies which have shown geographic variations in the incidence of cancer in various organs (Dunham and Bailar, 1968; Higginson, 1969) and the risks of cancer among migrants conforming within one or two generations to those of the adopted country (Haenszel *et al.*, 1972). As of yet, there is no consensus of scientific opinion regarding the exact percentage of cancer due to these factors, but the important fact remains that they, i.e., *physical*, *biological*, and *chemical factors* in the environment, contribute substantially to cancer incidence in the human population.

1. Physical Factors

Exposures to ultraviolet light and ionizing radiation have been shown to cause cancer (Jablon, 1975; Upton, 1975). Ultraviolet light from the sun is strongly associated with squamous cell carcinoma of the skin and, to lesser degrees, with basal cell epithelioma and malignant melanoma (Emmett, 1973). Ultraviolet light apparently interacts directly with nucleic acids of the skin cells (Upton, 1975). Ionizing radiation -- exposure to X-ray, radionucleides, or radiopharmaceuticals -- can have leukemogenic or other carcinogenic effects on the human (Jablon, 1975). Acute lymphoblastic and chronic myeloid leukemia may be caused by ionizing radiation; an increased incidence of tumors of the thyroid, respiratory tract, breast, gastrointestinal system (except stomach) and lymphosarcoma has been diagnosed in the population of Hiroshima and Nagasaki who received gamma and neutron radiation from the atomic explosions. In the case of the radium dial painters, radium isotope which localized in the bone eventually induced osterosarcomas and leukemia. But if one excludes skin cancer caused by prolonged exposure to the sun, a malady usually treatable, radiation presently plays a relatively small part, "a minute portion," in overall cancer incidence (Doll, 1977a; Upton, 1975).

2. Biological Factors

Other environmental agents that have been associated with cancer are microbes and parasites (Gross, 1978; Heath et al., 1975). DNA viruses -- particularly the Epstein-Barr virus (EBV) and herpes simplex virus type 2 (HSV-2)-- are suspect human oncogens. They have been associated with cancer in humans and have shown tumor formation in lower animals. However, proof of oncogenicity in humans has not been demonstrated for either virus. Suggestive evidence links the metabolic activity of intestinal bacterial flora to colon cancer and possibly breast cancer, urinary tract infection to gastric cancer, and infestation by the parasite Shistosoma haematobium with increased risk of malignancy. However, it is for these biological agents as it was for the viruses, proof of oncogenicity in the human is not yet conclusive (Heath et al., 1975). Consequently, whether biological agents cause cancer in the human is still an open question (Doll, 1977a).

3. Chemical Factors

The exogenous agents most often associated with the etiology of cancer are natural and synthetic chemicals. Boyland (1969) estimates that 90 percent of human cancers are evoked by chemical agents. In a recent study, cancers attributable to occupational factors -- particularly synthetic chemicals -- were estimated between 20 and 40 percent, a finding considerably higher than previous estimates (Bridbord *et al*., 1978). Epstein (1974) has suggested four broad categories of environmental chemicals:

(a) Natural chemicals which are normal dietary components, such as nitrates and nitrites;

(b) natural fungal or plant toxins in crops, such as aflatoxins and cycasins;

(c) complex organic and inorganic mixtures, for example the community air, water, and occupational pollutants, such as coke tar pitch volatiles;

(d) synthetic chemicals-agricultural chemicals, such as pesticides, fertilizers, food additives, fuel additives, household chemicals and industrial chemicals.

There is some controversy about which one of these four is the most significant category in the etiology of chemically-induced cancer. (Berg, 1977; Doll, 1977b; Weisburger, Cohen, and Wynder, 1977; Wynder and Gori, 1977; Devesa and Silverman, 1978; and Schneiderman, 1978). Nevertheless, it is with the synthetic and natural chemical substances, developed and produced by industry, which may present "an unreasonable risk of injury to health or the environment" (with the exception of pesticides and food additives covered by FIFRA and FFDCA respectively) that TSCA is primarily concerned. Knowledge of the carcinogenic activity of some of these chemicals, the interaction among environmental chemicals that can enhance tumorgenicity at low dose, the role of a variety of host factors in chemically-induced carcinogenesis, and the awareness of the potential for ever increasing exposure (often involuntary) of modern agricultural and industrial societies have caused the Congress and the EPA to focus on these chemicals.

F. Initiation, Promotion, and Other Chemical Interactions Which Enhance Oncogenic Potential of Low-Level Chemical Exposure

Relatively little is known about the response of a total population to lifelong oncogenic exposures which result from low-levels of both natural and synthetic oncogens in the environment (Saffiotti, 1977); the latent period associated with carcinogenesis can delay discovery of carcinogenic action for as much as forty years. Moreover, tumor type and incidence of chemically-induced cancers may be indistinguishable from cancers associated with other causes. However, exposure to even low-levels of carcinogens may pose serious risks, particularly under long-term exposure conditions, because of the chemical interactions that can occur in the human body. Cocarcinogenic interactions can significantly enhance the oncogenic effects of chemical substances administered at low doses to both human and laboratory animals.

1. Cocarcinogenesis

Cocarcinogenic action refers to the interaction of two or more factors which augment tumor induction when administered either together with or subsequent to a sub-optimal dose of a carcinogen (Berenblum, 1974). Berenblum classifies these different kinds of cocarcinogenic actions as follows:

1. Additive action, when the cocarcinogenic agent itself possesses definite carcinogenic activity.
2. Synergistic action, when the combined effect exceeds the summation of their separate actions.
3. 'Incomplete' carcinogenic action, responsible for only one phase of carcinogenesis, i.e. operating as initiator only or as promoter only.
4. Preparative action, by rendering the target organ or tissue more responsive to carcinogenic action.
5. Permissive influences of carcinogenic action, e.g. through solvent effect, or by influencing the rate of absorption of the carcinogen into the cell, or by affecting the metabolism of the carcinogen prior to its action, or by influencing its rate of detoxification and excretion, etc.
6. Influence on viral action, by favoring virus release from its hidden site: depressing the immune response of the animal, activating an incomplete virus, or by rendering the target organ responsive to the virus.
7. Conditional influence on the induced tumor during its previsible stage, by encouraging its continual growth, in the case of a hormone-dependent tumor, or by countering the immune resistance of the host to the tumor.

Of these various cocarcinogenic actions, incomplete carcinogenic action (initiation and promotion), synergistic action, and additive action have received considerable attention and will be at the focus of the following discussion.

a. Initiation and Promotion

Carcinogenesis is generally viewed as complex biochemical and cellular process which occurs in two stages: initiation followed by promotion. Initiation is the rapid induction of imperceptible and essentially irreversible changes in cells exposed to a carcinogen, (such as benzo(a)pyrene) which result in the production of transformed but dormant cells. Promotion is the stimulation of these transformed cells with an agent (such as phorbol esters) which ultimately causes them to grow and proliferate into progressively-growing tumors (Boutwell, 1974; Van Duuren, 1966). It has been found that promoters administered subsequent to a tracer dose of a carcinogenic polycylic aromatic hydrocarbon lead to extensive tumor formation on the skin of mice (Haddow, 1959, Hecker, 1971).

Evidence suggests, moreover, that the induction of lung cancer by exposure to cigarette smoke is due to relatively small amounts of the initiating carcinogen and larger amounts of promoting agents (Wynder and Hoffman, 1967, 1972; Van Duuren et al., 1971; Bock, 1972; Wynder and Mabushi, 1972). It has been found that cigarette smoke significantly enhances the development of lung cancer among uranium miners (Archer et al., 1976) and asbestos workers (Nicholson, 1976) and contains ingredients which are powerful promoters of polyclic aromatic carcinogens (Van Duuren et al., 1966). These findings show that three classes of carcinogens--polycyclic aromatic hydrocarbons, ionizing radiation, and asbestos--accompanied by promoter agents have a markedly enhanced tumor response in humans (Albert and Burns, 1977).

Because of this initiation-promotion interaction, low-level exposures to oncogens in an environment "loaded" with many other chemical substances, some of which may serve as promoters, may show a much larger tumor incidence than the actual dose would indicate and/or a shortened latent period for tumor development. In either case, the impact on human health could be serious.

b. Additives and Synergistic Interactions

Additive and synergistic (often called syncarcinogenic) interactions between chemicals and oncogens enhance the oncogen's potency to induce tumorgenesis. It has been shown that some exogenous chemicals (even with different chemical structures) which are not oncogenic alone at low doses may, when administered simultaneously or consecutively at the same low dose, react with the target organ and result in the development of tumors. Examples are the hepatotrophic oncogens: dimethyl- and diethylnitrosamine, nitrosomorpholine and dimethylaminobenzene (Schmähl, 1977). When these four chemicals are administered singly in subdivided doses, no heptomas develop even though the total dose is the same as for the combination study. But if the doses of the four chemicals are administered simultaneously, liver tumors develop, evidencing an additive effect. Montesano et al. (1974) reported a 31 percent incidence of squamous cell carcinomas of the tracheo-bronchial tract in hamsters with benz(a)pyrene followed by diethylnitrosamine under exposure conditions where neither carcinogen induced tumors alone. With regard to spontaneously occurring tumors, it would seem likely that exposure to environmental chemicals could synergistically or additively enhance these oncogenic processes (Crump et al., 1976).

2. Metabolic Activation of Chemicals and Carcinogenicity

Chemical oncogens may be divided into two broad classes: direct acting ultimate carcinogens and procarcinogens. Direct acting ultimate carcinogens, such as nitrogen or sulfur mustard, methyl methanesulfonate, bis-(chloromethyl) ether, and others, have a chemical structure which makes them inherently reactive, thus able to react directly with cellular and molecular receptors in the target cells. Most of the known environmentally important oncogens, such as polycyclic aromatic hydrocarbons, aromatic amines, amino azo dyes, aflatoxins, and others are procarcinogens; they require metabolic activation to reactive derivatives which can chemically interact with receptors in target cell(s) (Weisburger, 1978). For example, the liver's mixed function oxidase system is thought to activate the oncogens 3,4 benzypyrene, 2-acetylamino fluorene, and aflatoxin B (Felton and Nebert, 1975).

Examples of chemicals requiring and not requiring metabolic activation are found in the simple alkyl group (Fig. 2). Dimethylnitrosamine is metabolically dealkylated by mixed-function oxidases in the endoplasmic reticulum, forming the monoalkyl derivative which spontaneously decomposes to the reactive monoalkyldiazonium ions. On the other hand, N-methyl-N-nitrosourea does not require enzymatic activation; the reaction with water and other cellular nucleophiles results in formation of the monoalkyl derivative which likewise spontaneously decomposes to the monoalkyldiazonium ions (Miller, 1978).

In-vivo, the reactive chemical oncogen or metabolite has relatively electron-deficient atoms (electrophiles) that react with nucleophilic sites in the cell, i.e., atoms in the cell that easily share electrons. The macro-molecules, DNA, RNA, and protein in the cell in the target tissue have numerous nucleophilic sites to which the chemical derivatives bind, a process which correlates with the induction of transformed cells and the subsequent formation of neoplasia.

The crucial parameter in determining whether a given carcinogen is active under certain conditions, the degree and extent of its activity, the site it affects, and under some conditions the time required to elicit the effect, is the ratio of the enzyme-mediated activation-detoxification reactions (Weisburger and Williams, 1975). The introduction of exogenous chemicals into the human body, even at low levels, can modify the level of metabolic enzyme activity and as a consequence the activation-detoxification ratio. An increase in metabolic activation may enhance the oncogenic effects of low-dose chemicals. Given the myriad chemicals which occur in our environment, it is possible that the metabolic activation of some procarcinogens will be enhanced. Chemicals which have been shown to cause enzyme induction and thus enhance activation or detoxification reactions are polycyclic aromatic hydrogens and their quinones, phenobarbital, certain chlorocarbon pesticides, antioxidants, some hormones, and some dietary ingredients.

G. Identification of Chemicals with Chronic Health Effects: The Use of Epidemiology, Short-Term Tests for Predicting Oncogenicity, and Long-Term Chronic Toxicity Tests

Epidemiological studies may provide the most satisfactory data for investigating human health effects resulting from exposure to environmental chemicals. Such studies avoid the uncertainties of extrapolating experimental animal or *in vitro* system data to humans and the high doses of the bioassay to the probable low-dose of human exposure. However, epidemiology has inherent limitations. A program designed to prevent adverse health effects by identifying and regulating toxic chemicals must rely heavily on the direct testing of chemical substances for health effects. The following discussion reviews epidemiology, short-term tests, and long-term tests and explains why scientists at EPA presently view data developed by adequately designed and properly conducted long-term tests as the most reliable and timely means for evaluating chemicals for chronic health effects in human.

1. Epidemiology

Epidemiology has been defined as the study of the origin, nature, pathology, and prevention of diseases temporarily prevalent in a community or throughout a large area. Epidemiologists analyze human incidence and mortality rates, identifying significant changes in incidence or mortality for each disease; they compare inter- and intra-country rates and compute baseline or spontaneous rates for each disease and excessive rates which may be associated with environmental factors; and they attempt to establish a quantitative relationship between the dose of the environmental factors to which a population is exposed and the incidence of disease within the population.

Human epidemiology provides *post facto* information about the effects of chemicals on the human since it gathers health data from a population already exposed to a chemical. It is the most reliable data for human risk assessment and a very important method for detecting adverse health effects, for example, the increased incidence of bladder cancer among workers in benzidene (IARC, 1972) and B-napthylamine (IARC, 1974). But it is limited by a number of factors, some of which are the following:

a. Because of the long latency period for the development of most cancers and other chronic diseases in humans, epidemiological studies do not detect disease until after a population has been exposed and a significant number of its members contract the disease. Disease prevention, therefore, is effective only for later populations for whom exposure is limited or prevented by the findings of the earlier study. For cancer, where the latency period is 5-40 years, many years may pass before an effect is observed, and there is little that can be done to protect people previously exposed who have induced but as yet clinically unrecognizable tumors. In addition, since epidemiological studies are retrospective, they cannot be used to assess the risk of new chemical substances or substances which have only recently entered the environment.

b. People are exposed to chemicals in great varieties and concentrations in their job, diets, and surrounding environment. Singling out one factor as a cause of disease is difficult, especially in the case of cancer because of the possibility of synergistic effects and long latency periods of oncogens.

c. The population under study must be well-defined with regard to exposure; the inclusion of unexposed people in the study dilute it, causing an underestimate of risk.

d. When investigating chronic low-level environmental exposure, it is difficult to establish a control population, i.e., a major fraction of the population with little or no exposure.

e. The high degree of mobility in the U.S. society complicates the exposure pattern. People change doctors and hospitals often, making data collection difficult.

f. Individuals are resistent to participation in medical studies. Investigators are often unable to obtain medical histories and records.

g. Epidemiological studies are questionable when it comes to detecting toxic activity of environmental chemicals at low-levels in the atmosphere. Chronic toxic effects of chemicals may be too subtle to identify; they may be masked by other effects, attributed to other causes, or be one of a number of participatory causes for a disease process. Occupational exposure to chemicals such as 2-napthylamine, vinyl chloride, and asbestos gave rise, respectively, to rare cancers of the bladder, angiosarcomas of the liver, and mesotheliomas of the lung cavity. Such specific and rather rare tumors may be detected by epidemiological studies. But for environmental exposures to chemicals which induce a variety of common tumors, epidemiology probably will be unable to associate the malady with the caused factor (Bridbord et al. 1978; Berg, 1977).

To prevent chemically-induced chronic diseases, timely and reliable data on chronic health effects of chemical substances are essential. While epidemiological studies are extremely useful, they cannot be relied upon as the primary means of developing data EPA needs for regulatory actions concerning toxic substances. Testing of selected chemicals for oncogencity is essential.

2. Short-term Tests for Predicting Oncogenicity

Toxicologists generally agree that a common goal is the development of a battery of reliable short-term tests for predicting oncogenicity. Given the thousands of chemicals to which humans are exposed in the environment, most of which have not been tested adequately if at all for oncogenic effects, the need for quick, inexpensive short-term tests with high predictive value is obvious. These tests will be useful in identifying possible oncogenic chemicals and establishing their priority for testing in the long-term bioassay. Moreover, the data developed in the short-term tests will assist in the interpretation of the long-term effects data.

Although a variety of short-term *in vitro* tests are under development (tests based on unscheduled DNA synthesis in human skin fibroblasts; transformation of epithelial cells or fibroblasts *in vitro*, and others (Bridges, 1976; Miller, 1978; Purchase *et al*., 1976), the Salmonella/microsome mutation test developed by Ames and McCann appears to be the most popular (Ames, 1971; Ames *et al*., 1975; McCann and Ames, 1977); however, the *in vitro* cell transformation test has been shown to be an equally good indicator of oncogenicity (Purchase *et al*., 1976).

The Salmonella/microsome test and the *in vitro* cell transformation tests are relatively inexpensive, give quick results, and show sensitivity to very small chemical doses. Moreover, correlations with the results of long-term animal bioassay have been good. Validated against 175 chemicals whose oncogenic effects in animals are well-known, the Salmonella/microsome test detected 157 positives (90 percent); of 108 chemicals identified as non-oncogenic in animal tests, theSalmonella/microsome test detected 94 (87 percent) (McCann and Ames, 1977). In an evaluation performed by Purchase and colleagues (1976), again using compounds found oncogenic in animal tests, the Salmonella/microsome test was found 91 percent accurate in detecting oncogens and 93 percent accurate in detecting non-oncogens. And in this same evaluation, the cell transformation test, using neonatal Syrian hamster kidney fibroblasts, detected 91 percent of the carcinogens and 97 percent of the noncarcinogens.

A 90 percent reliability for the test chemicals still means, however, that a significant number of other chemicals could be misevaluated, given the extremely large and varied universe of chemicals either in the environment, underdevelopment, or pending distribution (Purchase _et al_., 1976). And too, until there is a rigorous method of validating the short-term test systems, perhaps along the lines suggested by Saffiotti (1978), the reliability of the short-term tests will remain in doubt.

EPA does not think that these short-term tests are presently capable of replacing the long-term bioassay for several important reasons. The Salmonella/microsome test detects the mutagenicity of a chemical, not its capacity to produce tumors, the "endpoint" of the long term bioassay. Insofar as chemically-induced carcinogenicity and mutagenicity correlate, the test may be a satisfactory indicator, but the extent of this correlation is not conclusively known; not all mutagens are demonstrable carcinogens and vice versa. Compounds such as metals, hormones, chrysene, urethan, and thioacetamide, all of whom have carcinogenic activity in intact animals but are not bacterial mutagens, would not be detected by the most sensitive and accurate Salmonella/microsome test (Tardiff, 1978).

For all short-term tests which use an _in vitro_ metabolic activating system, there is the possibility the _in vitro_ system does not mimic the whole animal or human system. Nor do the short-term tests mimic pharmacologic distribution of the chemical, macromolecular repair capabilities, immunological mechanisms or other factors of the mammalian system. Consequently, extrapolation of short-term _in vitro_ test data to man would be precarious. In fact, for the performance of human risk assessments there is presently no mechanism to extrapolate _in vitro_ data to man.

Two other potential weaknesses of the short-term tests concern mammalian aging processes and sex-linked differences. Since one characteristic of oncogenicity is the latent period, the mammalian aging process may be a significant parameter in oncogenicity testing. In his review of toxic drug reactions in old age, Zbinden (1973) indicates that there is a higher susceptibility of old people for toxic drug reaction because of the aging of tissue, decrease in enzyme activity, and general deterioration. Such conditions could possibly alter response to low-level exposure to environmental chemicals,and short-term tests are not sensitive to them. And sex-linked differences in response to chemical oncogens have also been shown in human and experimental animals; however, short-term tests presently are not capable of mimicing these differences.

Thus, the state-of-the-art and our understanding of short-term tests are not sufficiently advanced to allow such tests to be used in place of long-term tests.

3. Long-term Chronic Toxicity Tests

a. Oncogenicity Tests

A properly conducted, long-term oncogenicity test is presently the definitive test model for estimating the oncogenic risk of chemicals for humans (Miller, 1978; NAS/NRC, 1977; Page, 1977; Saffiotti, 1978). Having mammalian tumor-induction as its end-point, the oncogenicity bioassay is the only source of direct evidence (other than in the human) of chemically-induced tumors in the mammalian species. Moreover, of all test systems, it comes closest to mimicing human routes of exposure and metabolic/ pharmacologic processes which activate and distribute chemicals.

In keeping with the great importance of the oncogenicity bioassay, national and international advisory groups have reviewed the procedures for conducting long-term oncogenicity tests several times: NAS/NRC, 1969; WHO, 1969; Berenblum, 1969; FDA, 1971; Ad Hoc Committee on the Evaluation of Low Levels on Environmental Chemical Carcinogens, 1971. The National Cancer Institute has improved its Bioassay Program during the past few years and published its methods in the form of guidelines (Sontag, *et al*., 1976).

Recently, an IARC/WHO ad hoc work group revised its assessment criteria for evaluating chemically-induced oncogenicity in humans and/or experimental animals and concluded:

> In the presence of appropriate experimental carcinogenicity data and in the absence of adequate human data, it is reasonable to regard chemicals for which there is 'strong evidence' of carcinogenicity (i.e., unquestionable production of malignant tumors in animals) as if they were carcinogenic to humans (Tomatis *et al*., 1978).

Most types of human cancers can now be chemically-induced in animals in the oncogenicity bioassay. Animal models for assessing carcinogenicity include: bronchogenic lung cancer; carcinoma of the larynx; large bowel cancer; carcinoma of the pancreas; kidney carcinoma; urinary bladder carcinoma, and mammary cancer (Saffiotti, 1978). And virtually all oncogens known to be active in humans have also been shown positive in animal tests.

The long-term oncogenicity bioassay is not always an optimal test system. For example, some critics emphasize that substances used in animals test systems are usually specific agents, whereas, humans are exposed to multiple environmental stresses. This same criticism applies, of course, to short-term tests. Other critics assert that the high doses administered to test animals are not comparable to the low-levels of exposure experienced in the environment. However high dosing is necessary to assure the sensitivity of the test, moreover, it has been shown that high dosing does not necessarily cause false positive results (Innes *et al.*, 1969). (Cf. discussion of dose in Section II.B.5a). Some commentators contend that extrapolation of laboratory data from test animals to man can be very difficult because interspecies variations in the metabolism of chemicals, in the enzyme systems, in life-span are variables whose consequences are largely unknown for testing. In this regard, the National Academy of Sciences/National Research Council's report on Pest Control (1975) concluded:

> As a working hypothesis, in the absence of countervailing evidence for the specific agent in question....it appears reasonable to assume that the lifetime cancer incidence induced by chronic exposure in man can be approximated by the lifetime incidence induced by similar exposure in laboratory animals at the same total dose per body weight.

EPA agrees that evidence sufficiently indicates that results shown in the long-term bioassay can predict the human risk and should be used for the identification and removal of cancer causing chemicals (Rall, 1978; Tomatis, 1974; Tomatis, 1977). For further discussion, consult Section II of this document.

b. Non-Oncogenic Chronic Toxicity Tests

Given the large volume of chemicals in commerce and under development which require testing, the large investment of time and money in the traditional, long-term bioassay, the possibly serious impact that toxic chemicals in the environment may have on human health, and the awareness that epidemiological studies cannot provide primary data in most cases, scientists have attempted to design an optimal chronic toxicity test system which will provide adequate toxicologic information while reducing the investments of time and money for each chemical test.

Short-term (3-4 months) chronic tests have been proposed by Weil and McCollister (1963) and McNamara (1976). Although such studies are useful in establishing no-effect dose levels for environmental chemicals, scientists at EPA do not think such tests provide adequate data for Section 4 test requirements.

Since humans are exposed to synthetic chemicals in the environment for probably a lifetime, chronic toxicity tests for assessing their health effect should be for a comparable period of time--lifetime or near-lifetime for studies in laboratory animals (Loomis, 1968; NAS, 1975).

Testing for this period of time in animals (24-30 months) allows observation of toxic effects related to the aging process; age related factors such as altered tissue sensitivity, changing metabolic and physiological capability, and spontaneous disease, which can influence the degree and nature of toxic responses, can be assessed (WHO, 1978). Moreover, with long-term testing, chemicals may produce different toxic responses with repeated dosing; different metabolic pathways may become involved; bioaccumulation of the chemical in the tissues of the host may occur. Only with long-term dosing can these responses as well as effects on the longevity of the animal's life be monitored.

In short, the non-oncogenic chronic toxicity test is the most reliable means to establish a no-observed-adverse- effects level which can then be used to define the lifetime "acceptable daily intake" (ADI) of a chemical for humans.

Appendix A to Section I

Definitions

In this document, terminology specific to cancer is consistent with definitions and usage found in the cancer literature. To assure a common understanding of key terms, however, the following definitions and references which characterize the disease are provided.

Cancer -- an extremely complex, multistaged disease process or group of disease processes which gives rise to malignant tumors (Anderson and Scotti, 1976; Berenblum, 1974; Weinstein et al., 1975).

Tumor -- a neoplasm, an abnormal mass of tissue developed by progressive growth and proliferation of transformed cells at a rate uncoordinated with contiguous normal cells. Growth continues after cessation of the triggering stimulus. Although composed of cells and intercellular substances apparently similar to those found in embryonic and mature tissues, the tissue mass is characterized by growth activity rather than function; function may, however, not be lacking. More than 270 types of human neoplasms, including cancer, have been recognized and defined histologically, each of which may be distinct in its behavior. Tumors are generally classified as benign or malignant, depending on their biological characteristics (Anderson and Scotti, 1976; Berenblum, 1974; Willis, 1967.

Benign Tumor -- a tissue mass that is usually slow-growing and expansive but which after reaching a certain size may cease to expand. A benign tumor may press against or push aside normal tissues, but its cells do not invade adjoining tissue or metastasize. The tumor appears as a circumscribed, well-demarcated growth, usually surrounded by a fibrous capsule. Histologically, benign tumors are composed of well-differentiated, mature tissue closely imitating the normal tissue of their origin (Anderson and Scotti, 1976). Although some benign tumors do not endanger the life of the host, if they are situated so as to interfer with some vital organ, cause hemorrhage, or promote unregulated hormone production, they can be life-threatening (Berenblum, 1974). Moreover, some benign tumors may become malignant and thus of serious concern (Cf. discussion under "Oncogenesis).

Malignant Tumor -- a cancer, a tissue mass that usually grows by expansion and invasion of surrounding tissue. Generally unencapsulated and poorly demarcated, malignant tumors often spread by local invasion of surrounding tissue or metastasis, i.e., transformed cells break off from the tumor mass and migrate through blood or the lymphatic system and establish colonies in distant organs. Histologically, the cells and structural organization of malignant tumors generally exhibit an inadequate maturation and, to varying degrees, a lack of differentiation so pronounced that often the tissue of origin is difficult to identify. Unless the malignant tumor is removed or its cells killed, it usually causes the death of the host. Approximately 100 different types of cancer have been classified (Anderson and Scotti,1976; Berenblum, 1974).

Carcinogenesis -- the production of a cancer, or malignant tumor. Although most cancers originate in epithelial tissues, -- particularly lining epithelial of the skin, hollow organs, and respiratory, digestive and genitourinary systems -- malignant neoplasms other than carcinoma are included in the definition of carcinogenesis, i.e., neoplasms which arise in the connective tissue (sarcomas) and in the blood-forming tissues (leukemias). Moreover, since the distinction between malignant neoplasms and benign neoplasms is in some cases very difficult to judge (see discussion below), the inclusion of benign neoplasms in the definition of carcinogenesis, not withstanding the fact that a cancer is not benign, has been suggested: "Carcinogenesis is the generation of benign and malignant neoplasia in the broadest possible sense including generation of sarcomata and leukemia" (Hecker, 1976).

Oncogenesis -- (from the Greek onkos, meaning bulk or mass and the Latin genere, meaning to make or create) the production of tumors in the broadest possible sense, both benign and malignant.

Since EPA's employment of the term oncogenesis may be controversial, a brief description of the Agency's rationale follows.

In the past, a tumorigen, when defined as an agent which induced only benign tumors, was considered less dangerous than a carcinogen, a malignant tumor-inducing agent. This classification is unsatisfactory, however. The conversion of benign papillomas of the skin into squamous carcinomas which invade and metastasize has been shown (Berenblum, 1974); moreover, benign growths such as leukoplakea of the oral cavity, ostertis deformans (a bone disease), and polyps in the digestive tract (particularly villous papillomas) appear to become malignant (Levin et al., 1974). The International Agency for Research on Cancer (IARC) has concluded:

> Many chemicals induce both benign and malignant tumors; few instances are recorded in which only benign neoplasms are induced by chemicals that have been studied extensively. Benign tumors may represent a state in the evolution of a malignant neoplasm or they may be 'end-points' which do not readily undergo transition to malignant neoplasm. If a substance is found to induce only benign neoplasms in experimental animals, the chemical should be suspected of being a carcinogen and requires investigation (Tomatis et al., 1978).

The IARC position generally reflects scientific opinion on the issue (Mrak Commission Report, 1969). In agreement, EPA has taken the position that for the purposes of performing risk assessment for human health-effects, all tumorigens must be considered as potential carcinogens; "any evidence of tumorigenic activity in animals is a signal that the agent is a potential human carcinogen" (Albert, et al., 1977; EPA, 1976).

Consequently, the term oncogenesis and its derivatives seem most appropriate for describing the disease process which includes benign and malignant neoplasms, including the generation of sarcomata and leukemia. An oncogen then is a chemical, physical, or biological agent which induces either benign or malignant neoplasms, or both.

Irreversibility of the Oncogenic Effect -- refers to the irreversible change of a normal cell to a neoplastic cell effected by an oncogen. The neoplastic cells are self-replicating, i.e., clone neoplastic daughter cells which in turn replicate neoplastic cells, and this process continues, forming the tumor. Once transformation of the target cell has occurred, the cells are capable of irreversible autonomous growth regardless of whether exposure to the oncogen continues.

Latent Period - a period of time between exposure to an oncogen and manifestation of a tumor -- estimated to be 5 to 40 years in the human.

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TABLES AND FIGURES

SECTION I

Table 1

Chemicals or industrial processes associated with cancer induction in humans: comparison of target organs and main routes of exposure in animals and humans

	Humans			Animals		
Chemical or industrial process	Main type of exposure[a]	Target organ	Main route of exposure[b]	Animal	Target organ	Route of exposure
1. Aflatoxins	Environmental, occupational[c]	Liver	p.o., inhalation[c]	Rat	Liver, stomach, colon, kidney	p.o.
				Fish, duck, marmoset, tree shrew, monkey	Liver	p.o
				Rat	Liver, trachea	i.t.
					Liver	i.p.
				Mouse, rat	Local	s.c. injection
				Mouse	Lung	i.p.
2. 4-Aminobiphenyl	Occupational	Bladder	Inhalation, skin, p.o.	Mouse, rabbit, dog	Bladder	p.o.
				Newborn mouse	Liver	s.c. injection
				Rat	Mammary gland, intestine	s.c. injection
3. Arsenic compounds	Occupational, medicinal, and environmental	Skin, lung, liver[c]	Inhalation, p.o., skin	Mouse, rat, dog	Inadequate, negative	p.o.
				Mouse	Inadequate, negative	Topical, i.v.
4. Asbestos	Occupational	Lung, pleural cavity, gastrointestinal tract	Inhalation, p.o.	Mouse, rat, hamster, rabbit	Lung, pleura	Inhalation or i.t.
				Rat, hamster	Local	Intrapleural
				Rat	Local	i.p., s.c. injection
					Various sites[c]	p.o.
5. Auramine (manufacture of)	Occupational	Bladder	Inhalation, skin, p.o.	Mouse, rat	Liver	p.o.
				Rabbit, dog	Negative	p.o.
				Rat	Local, liver, intestine	s.c. injection
6. Benzene	Occupational	Hemopoietic system	Inhalation, skin	Mouse	Inadequate	Topical, s.c. injection
7. Benzidine	Occupational	Bladder	Inhalation, skin, p.o.	Mouse	Liver	s.c. injection
				Rat	Liver	p.o.
					Zymbal gland, liver, colon	s.c. injection
				Hamster	Liver	p.o.
				Dog	Bladder	p.o.
8. Bis(chloromethyl) ether	Occupational	Lung	Inhalation	Mouse, rat	Lung, nasal cavity	Inhalation
				Mouse	Skin	Topical
					Local, lung	s.c. injection
				Rat	Local	s.c. injection
9. Cadmium-using industries (possibly cadmium oxide)	Occupational	Prostate, lung[c]	Inhalation, p.o.	Rat	Local, testis	s.c. or i.m. injection
10. Chloramphenicol	Medicinal	Hemopoietic system	p.o., injection	No adequate tests		
11. Chloromethyl methyl ether (possibly associated with bis(chloromethyl) ether	Occupational	Lung	Inhalation	Mouse	Initiator	Skin
					Lung[c]	Inhalation
					Local, lung[c]	s.c. injection
				Rat	Local[c]	s.c. injection
12. Chromium (chromate-producing industries)	Occupational	Lung, nasal cavities[c]	Inhalation	Mouse, rat	Local	s.c., i.m. injection
				Rat	Lung	Intrabronchial implantation

Table 1 – Continued

	Humans			Animals		
Chemical or industrial process	Main type of exposure[a]	Target organ	Main route of exposure[b]	Animal	Target organ	Route of exposure
13. Cyclophosphamide	Medicinal	Bladder	p.o., injection	Mouse	Hemopoietic system, lung	i.p., s.c. injection
					Various sites	p.o.
				Rat	Bladder[c]	i.p.
					Mammary gland	i.p.
					Various sites	i.v.
14. Diethylstilbestrol	Medicinal	Uterus, vagina	p.o.	Mouse	Mammary	p.o.
				Mouse	Mammary, lymphoreticular, testis	s.c. injection, s.c. implantation
					vagina	Local
				Rat	Mammary, hypophysis[c] bladder	s.c. implantation
				Hamster	Kidney	s.c. injection, s.c. implantation
				Squirrel monkey	Uterine serosa	s.c. implantation
15. Hematite mining (? radon)	Occupational	Lung	Inhalation	Mouse, hamster, guinea pig	Negative	Inhalation, i.t.
				Rat	Negative	s.c. injection
16. Isopropyl oils	Occupational	Nasal cavity, larynx	Inhalation	No adequate tests		
17. Melphalan	Medicinal	Hemopoietic system	p.o., injection	Mouse	Initiator	Skin
					Lung, lymphosarcomas	i.p.
				Rat	Local	i.p.
18. Mustard gas	Occupational	Lung, larynx	Inhalation	Mouse	Lung	inhalation, i.v.
					Local, mammary	s.c. injection
19. 2-Naphthylamine	Occupational	Bladder	Inhalation, skin, p.o.	Hamster, dog, monkey	Bladder	p.o.
				Mouse	Liver, lung	s.c. injection
				Rat, rabbit	Inadequate	p.o.
20. Nickel (nickel refining)	Occupational	Nasal cavity, lung	Inhalation	Rat	Lung	Inhalation
				Mouse, rat, hamster	Local	s.c., i.m. injection
				Mouse, rat	Local	i.m. implantation
21. *N,N*-Bis(2-chloroethyl)-2-naphthylamine	Medicinal	Bladder	p.o.	Mouse	Lung	i.p.
				Rat	Local	s.c. injection
22. Oxymetholone	Medicinal	Liver	p.o.	No adequate tests		
23. Phenacetin	Medicinal	Kidney	p.o.	No adequate tests[d]		
24. Phenytoin	Medicinal	Lymphoreticular tissues	p.o., injection	Mouse	Lymphoreticular tissues	p.o., i.p.
25. Soot, tars, and oils	Occupational, environmental	Lung, skin (scrotum)	Inhalation, skin	Mouse, rabbit	Skin	Topical
26. Vinyl chloride	Occupational	Liver, brain,[c] lung[c]	Inhalation, skin	Mouse, rat	Lung, liver, blood vessels, mammary, Zymbal gland, kidney	Inhalation

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[a] The main types of exposures mentioned are those by which the association has been demonstrated; exposures other than those mentioned may also occur.

[b] The main routes of exposure given may not be the only ones by which such effects could occur.

[c] Indicative evidence.

[d] The induction of tumors of the nasal cavities in rats given phenacetin has been reported recently (S. Odashima, personal communication, 1977).

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Table 2

List of chemicals for which there is some evidence of carcinogenicity in experimental animals only or for which the data were inadequate for evaluation of the presence or absence of carcinogenicity (IARC monographs, Volumes 1 to 16

For the 26 compounds evaluated as carcinogenic to humans. see Table 1.

1. Acetamide*[a]
2. Acridine orange
3. Acriflavinium chloride
4. Actinomycins*
5. Adriamycin
6. Aldrin
7. Amaranth
8. 5-Aminoacenaphthene
9. *p*-Aminoazobenzene*
10. *o*-Aminoazotoluene*
11. *p*-Aminobenzoic acid
12. 2-Amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole*
13. 4-Amino-2-nitrophenol
14. Amitrole*
15. Aniline
16. Anthranilic acid
17. Apholate
18. Aramite*
19. Arsenic trioxide
20. Aurothioglucose*
21. Azaserine*
22. Aziridine*
23. 2-(1-Aziridinyl)ethanol*
24. Aziridyl benzoquinone*
25. Azobenzene*
26. Barium chromate
27. Benz(*a*)acridine*
28. Benz(*c*)acridine*
29. Benzo(*b*)fluoranthene*
30. Benzo(*j*)fluoranthene*
31. Benzo(*a*)pyrene*
32. Benzo(*e*)pyrene*
33. Benzyl chloride*
34. Benzyl violet 4B*
35. Beryllium*
36. Beryllium oxide*
37. Beryllium phosphate*
38. Beryllium sulfate*
39. Beryl ore*
40. BHC (technical grades)*
41. Bis(1-aziridinyl)morpholinophosphine sulfide*
42. Bis(chloroethyl) ether*
43. 1,2-Bis(chloromethoxy)ethane*
44. 1,4-Bis(chloromethoxymethyl)benzene*
45. Blue VRS*
46. Brilliant blue FCF*
47. 1,4-Butanediol dimethane-sulfonate (Myleran)*
48. *β*-Butyrolactone*
49. *γ*-Butyrolactone
50. Cadmium acetate
51. Cadmium chloride*
52. Cadmium powder*
53. Cadmium sulfate*
54. Cadmium sulfide*
55. Calcium arsenate
56. Calcium chromate*
57. Cantharidin*
58. Carbaryl
59. Carbon tetrachloride*
60. Carmoisine
61. Catechol
62. Chlorambucil*
63. Chlorinated dibenzodioxins
64. Chlormadinone acetate*
65. Chlorobenzilate*
66. Chloroform
67. Chloropropham
68. Chloroquine
69. *p*-Chloro-*o*-toluidine (hydrochloride)
70. Cholesterol
71. Chromic chromate*
72. Chromium acetate
73. Chrysene*
74. Chrysoidine*
75. C.I. Disperse Yellow 3
76. Cinnamyl anthranilate
77. Citrus red No. 2*
78. Copper 8-hydroxyquinoline
79. Coumarin*
80. Cycasin*
81. Cyclochlorotine*
82. 2,4-D and esters
83. Daunomycin*
84. D & C Red No. 9
85. Dichlorodiphenyldichloroethane (DDD)
86. 1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE)
87. DDT*
88. Diacetylaminoazotoluene
89. *N,N*-Diacetylbenzidine*
90. Diallate*
91. 2,4-Diaminoanisole (sulfate)
92. 4,4'-Diaminodiphenyl ether*
93. 1,2-Diamino-4-nitrobenzene
94. 1,4-Diamino-2-nitrobenzene
95. 2,6-Diamino-3-(phenylazo)-pyridine (hydrochloride)
96. 2,4-Diaminotoluene*
97. 2.5-Diaminotoluene (sulfate)
98. Diazepam
99. Diazomethane*
100. Dibenz(*a,h*)acridine*
101. Dibenz(*a,j*)acridine*
102. Dibenz(*a,h*)anthracene*
103. Dibenzo(*c,g*)carbazole*
104. Dibenzo(*h,rst*)pentaphene*
105. Dibenzo(*a,e*)pyrene*
106. Dibenzo(*a.h*) pyrene*
107. Dibenzo(*a,i*)pyrene*
108. Dibenzo(*a.l*)pyrene*
109. 1,2-Dibromo-3-chloropropane*
110. Dibutylnitrosamine*
111. *o*-Dichlorobenzene
112. *p*-Dichlorobenze
113. 3,3'-Dichlorobenzidine*
114. *trans*-Dichlorobutene
115. 3,3'-Dichloro-4,4'-diamino-diphenyl ether*
116. Dieldrin*
117. Diepoxybutane*
118. 1,2-Diethylhydrazine*
119. Diethylnitrosamine*
120. Diethyl sulfate*
121. Diglycidyl resorcinol ether
122. Dihydrosafrole*
123. Dimethisterone
124. Dimethoxane*
125. 3,3'-Dimethoxybenzidine*
126. *p*-Dimethylaminoazobenzene*
127. *p*-Dimethylaminobenzenediazo-sodium sulfonate
128. *trans*-2-[(Dimethylamino)methylamino]-5-[2-(5-nitro-2-furyl)vinyl]-1,3,4-oxadiazole*
129. 3,3'-Dimethylbenzidine*
130. Dimethylcarbamoyl chloride*
131. 1,1-Dimethylhydrazine*
132. 1,2-Dimethylhydrazine*
133. Dimethylnitrosamine*
134. Dimethyl sulfate*
135. Dinitrosopentamethylenetetramine
136. 1,4-Dioxane*
137. 2,4'-Diphenyldiamine
138. Disulfiram
139. Dithranol*
140. Dulcin
141. Endrin
142. Eosin (disodium salt)
143. Epichlorohydrin*
144. 1-Epoxyethyl-3,4-epoxycyclohexane*
145. 3,4-Epoxy-6-methylcyclohexylmethyl-3,4-epoxy-6-methyl carboxylate*
146. *cis*-9,10-Epoxystearic acid
147. Estradiol mustard*
148. Ethinylestradiol*
149. Ethionamide*
150. Ethylene dibromide*
151. Ethylene oxide
152. Ethylene sulfide*
153. Ethylenethiourea*
154. Ethyl methanesulfonate*
155. Ethyl Selenac
156. Ethyl Tellurac
157. Ethynodiol diacetate*
158. Evans blue*
159. Fast green FCF*
160. Ferbam
161. 2-(2-Formylhydrazino)-4-(5-nitro-2-furyl)thiazole*
162. Fusarenon-X
163. Glycidaldehyde*
164. Glycidyl oleate
165. Glycidyl stearate
166. Griseofulvin*
167. Guinea green B*
168. Heptachlor
169. Hexamethylphosphoramide*
170. Hycanthone (mesylate)*
171. Hydrazine*
172. Hydroquinone
173. 4-Hydroxyazobenzene
174. 8-Hydroxyquinoline
175. Hydroxysenkirkine
176. Indeno(1,2,3-*cd*)pyrene*
177. Iron dextran*
178. Iron dextrin*
179. Iron oxide
180. Iron-sorbitol-citric acid complex
181. Isatidine*
182. Isonicotinic acid hydrazide*
183. Isopropyl alcohol
184. Isosafrole*
185. Jacobine
186. Lasiocarpine*
187. Lead acetate*
188. Lead arsenate
189. Lead carbonate
190. Lead chromate
191. Lead phosphate*
192. Lead subacetate*
193. Ledate
194. Light green SF*
195. Lindane*
196. Luteoskyrin*
197. Magenta*
198. Maleic hydrazide*
199. Maneb
200. Mannomustine (dihydrochloride)*
201. Medphalan
202. Medroxyprogesterone acetate*
203. Merphalan*
204. Mestranol*
205. Methoxychlor
206. 2-Methylaziridine*
207. Methylazoxymethanol acetate*
208. Methyl carbamate
209. *N*-Methyl-*N*,4-dinitrosoaniline*
210. 4,4'-Methylenebis(2-chloroaniline)*
211. 4,4'-Methylenebis(2-methylaniline)*
212. 4,4'-Methylenedianiline
213. Methyl iodide*
214. Methyl methanesulfonate*
215. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine*
216. Methyl red
217. Methyl Selenac
218. Methylthiouracil*
219. Metronidazole*
220. Mirex*
221. Mitomycin C*
222. Monocrotaline*
223. Monuron*
224. 5-(Morpholinomethyl)-3-[(5-nitrofurfurylidene)-amino]-2-oxazolidinone*
225. 1-Naphthylamine*
226. Native carrageenans*
227. Nickel carbonyl*
228. Nickelocene*
229. Nickel oxide*
230. Nickel powder*
231. Nickel subsulfide*
232. Niridazole*
233. 5-Nitroacenaphthene*
234. 4-Nitrobiphenyl*
235. Nitrofuraldehyde semicarbazone
236. 1-[(5-Nitrofurfurylidene)amino]-2-imidazolidinone*
237. *N*-[4-(5-Nitro-2-furyl)-2-thiazolyl]-acetamide*

Table 2 —*Continued*

238. Nitrogen mustard (hydrochloride)*
239. Nitrogen mustard *N*-oxide (hydrochloride)*
240. Nitrosoethylurea*
241. Nitrosomethylurea*
242. *N*-Nitroso-*N*-methylurethan*
243. Norethisterone*
244. Norethisterone acetate*
245. Norethynodrel*
246. Norgestrel
247. Ochratoxin A
248. 17β-Oestradiol*
249. Oestriol
250. Oestrone*
251. Oil orange SS*
252. Orange I*
253. Orange G
254. Oxazepam*
255. Oxyphenbutazone
256. Parasorbic acid*
257. Patulin*
258. Penicillic acid*
259. Phenicarbazide*
260. Phenobarbital sodium*
261. Phenoxybenzamine*
262. Phenylbutazone
263. *m*-Phenylenediamine (hydrochloride)
264. *p*-Phenylenediamine (hydrochloride)
265. *N*-Phenyl-2-naphthylamine*
266. Polychlorinated biphenyls*
267. Ponceau MX*
268. Ponceau 3R*
269. Ponceau SX
270. Potassium arsenite
271. Potassium bis(2-hydroxyethyl)-dithiocarbamate*
272. Progesterone*
273. Pronetalol hydrochloride*
274. 1,3-Propanesultone*
275. Propham
276. β-Propiolactone*
277. *n*-Propyl carbamate*
278. Propylene oxide*
279. Propylthiouracil*
280. Pyrimethamine*
281. *p*-Quinone
282. Quintozene*
283. Reserpine
284. Resorcinol
285. Retrorsine*
286. Rhodamine B*
287. Rhodamine 6G*
288. Riddelliine
289. Saccharated iron*
290. Safrole*
291. Scarlet red
292. Selenium compounds
293. Semicarbazide (hydrochloride)*
294. Seneciphylline
295. Senkirkine
296. Sodium arsenate
297. Sodium arsenite
298. Sodium dichromate
299. Sodium diethyldithiocarbamate
300. Sterigmatocystin*
301. Streptozotocin*
302. Strontium chromate*
303. Styrene oxide
304. Succinic anhydride*
305. Sudan I*
306. Sudan II*
307. Sudan III
308. Sudan brown RR
309. Sudan red 7B
310. Sunset yellow FCF
311. 2,4,5-T and esters
312. Tannic acid*
313. Terpene polychlorinates*
314. Testosterone*
315. Tetraethyl & tetramethyl lead
316. Thioacetamide*
317. 4,4′-Thioaniline*
318. Thiouracil*
319. Thiourea*
320. Thiram
321. *o*-Toluidine (hydrochloride)
322. Trichloroethylene*
323. Trichlorotriethylamine hydrochloride
324. Triethylene glycol diglycidyl ether*
325. Tris(aziridinyl)-*p*-benzoquinone*
326. Tris(1-aziridinyl)phosphine oxide
327. Tris(1-aziridinyl)phosphine sulfide*
328. 2,4,6-Tris(1-aziridinyl)-*s*-triazine*
329. 1,2,3-Tris(chloromethoxy)propane*
330. Tris(2-methyl-1-aziridinyl)phosphine oxide
331. Trypan blue*
332. Uracil mustard*
333. Urethan*
334. Vinyl cyclohexane
335. 2,4-Xylidine (hydrochloride)
336. 2,5-Xylidine (hydrochloride)
337. Yellow AB
338. Yellow OB*
339. Zectran
340. Zinc chromate hydroxide*
341. Zineb
342. Ziram

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* Asterisk, chemicals for which there is some evidence of carcinogenicity in experimental animals only.

Tomatis *et al.*, 1978. Permission to reproduce Table 2 granted by publisher *Cancer Research*

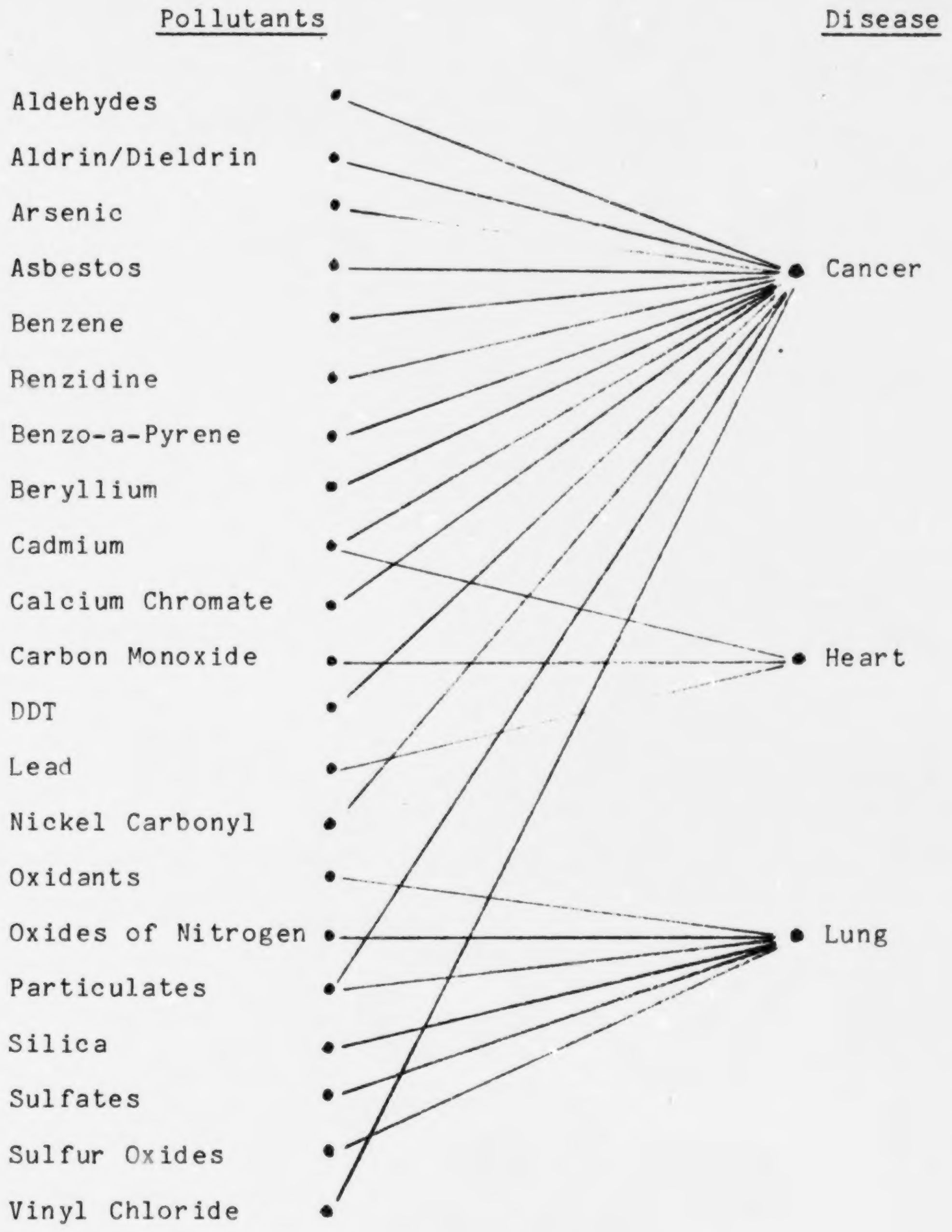
Pollutants
Disease
Aldehydes
Aldrin/Dieldrin
Arsenic
Asbestos
Benzene
Benzidine
Benzo-a-Pyrene
Beryllium
Cadmium
Calcium Chromate
Carbon Monoxide
DDT
Lead
Nickel Carbonyl
Oxidants
Oxides of Nitrogen
Particulates
Silica
Sulfates
Sulfur Oxides
Vinyl Chloride
Cancer
Heart
Lung

Figure 1. Known or Suspected Links Between Selected Pollutants and Disease (Task Force on Environmental Cancer and Heart and Lung Disease, 1978). Permission to use figure granted by EPA.

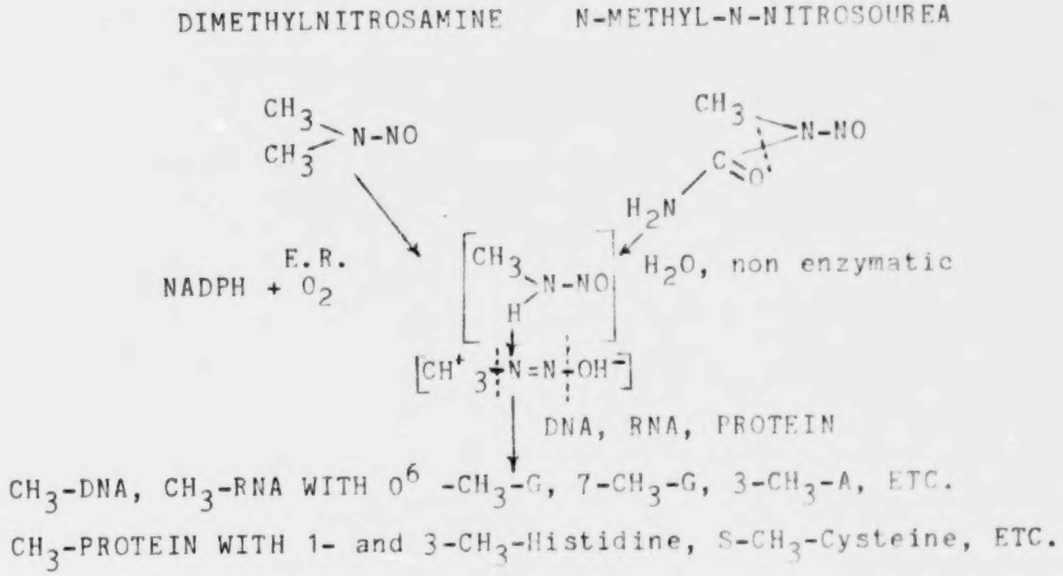
DIMETHYLNITROSAMINE
N-METHYL-N-NITROSOUREA
CH3 CH3 N-NO
CH3 N-NO C O H2N
E.R.
NADPH + O2
CH3 N-NO H
H2O, non enzymatic
CH+3 N=N OH−
DNA, RNA, PROTEIN
CH3-DNA, CH3-RNA WITH O6 -CH3-G, 7-CH3-G, 3-CH3-A, ETC.
CH3-PROTEIN WITH 1- and 3-CH3-Histidine, S-CH3-Cysteine, ETC.

Figure 2. The in vivo conversion of dimethylnitrosamine and of N-methyl-N-nitrosourea to a reactive electrophile and its reaction with cellular macromolecules.

Miller, 1978. Permission to use Figure 2 granted by publisher of Cancer Research.

II. Scientific Aspects of the Proposed Chronic Health Effects Test Standards

A. Overview of Proposed Standards

1. Objectives
2. Need for Standards
3. Summary of Standards

B. Study Design Issues

1. Introduction

2. Test Species, Strain, and Sex

   Objective
   - a. Test Species
     - i. Oncogenicity Studies
     - ii. Non-oncogenic Chronic Toxicity Studies
     - iii. Combined Chronic Toxicity Studies
     - iv. Alternative Species
   - b. Test Strain
   - c. Sex

3. Age at Start of Test

   Objective
   - a. Weanlings
   - b. In Utero
   - c. Neonatals

4. Number of Animal/Test Group

   Objective
   - a. Number
   - b. Randomization

5. Number of Dose Levels and Dose Selection

   Objective
   - a. Oncogenicity Studies
   - b. Non-oncogenic Chronic Toxicity Studies
   - c. Combined Toxicity Studies

6. Controls

   Objective
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   b. Positive Controls
   c. Historical Controls

7. Route(s) of Exposure
   Objective
   Discussion

8. Period of Exposure and Observation

   Objective
   a. Period of Exposure
      i. Oncogenicity Test Standards
      ii. Non-oncogenic Chronic Toxicity Test Standards
         A. Rodents
         B. Nonrodents

   b. Period of Observation

C. Study Conduct Issues

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   b. Clinical Chemistry

3. Pathology Procedures

   Objectives
   a. General
   b. Gross Necropsy
   c. Microscopic Examination

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1. Final Report
2. Interim Reports

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2. Personnel
3. Animal Care and Facility
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Objective
a. Diet
b. Standardization of Diet

5. Contaminant Analysis Requirements
Objective
Discussion

6. Safety and Health Standards

F. References

II-1.C

II. Scientific Aspects of the Proposed Chronic Health Effects Test Standards

A. OVERVIEW OF TEST STANDARDS

1. Objectives

Method of choice in a chronic toxicity study depends on objective(s) of the study and intended use of results (Weisburger and Weisburger, 1967; Arcos, Argus, and Wolf, 1968; NAS, 1975; Page 1977a; WHO, 1978a). Based on this, three general types of chronic toxicity studies have evolved: (1) studies to determine whether one or a combination of chemicals possesses toxic activity; (2) studies to determine structure-activity relationships; and (3) studies to define the mechanism(s) of action (Arcos, Argus, and Wolf, 1968). The objective of chronic toxicity test standards to be promulgated under section 4(b) of TSCA is to test a variety of high concern, individual environmental chemicals for chronic toxicity[1]. The ultimate use of test data will be to help evaluate possible risks to humans due to these chemicals.

Part 772, Subpart D, Chapter I of Title 40 of the Code of Federal Regulations will prescribe three chronic health effects test standards: Section 772.113-2 will prescribe oncogenic effects test standards; Section 772.113-3 non-oncogenic chronic effects test standards; and Section 772.113-4 combined chronic effects test standards. The main objective of studies carried out under Section 772.113-2 will be to determine the oncogenic potential of chemicals; that of Section 772.113-3 will be to determine chronic toxicity potential other than oncogenicity, and that of Section 772.113-4 will be to determine any chronic toxicity potential including oncogenicity.

The Agency is proposing combined chronic effects standards because it anticipates that certain chemicals will be required to undergo both oncogenicity and non-oncogenic chronic toxicity testing. In order to maximize efficient use of test animals, laboratory facilities and personnel resources, the combined test may be used

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1/ The proposed test standards are not designed to study synergistic effects such as promotion and cocarcinogenesis, structure-activity relationships or mechanism(s) of action. However, because data on such effects and relationships are relevant to the objectives of chronic toxicity testing, if such data are developed, they must be submitted to EPA. Such data may be required for certain chemicals as specified in specific Section 4(a) test rules.

instead of separate studies for oncogenic and non-oncogenic chronic effects. The proposed combined test meets the standards necessary for assessing both oncogenicity/ non-oncogenic chronic toxicity (see parts B and C of this section) while at the same time decreasing the cost and resource needs of such testing. As discussed in Section III of this document, an oncogenic effects test is estimated to cost approximately \$400,000[1]; a non-oncogenic effects test about \$500,000[1]; and a combined chronic effects test \$800,000[1]. Thus there will be a savings of around \$150,000 if both types of effects must be determined and the combined chronic effects test is used.

2. Need for Standards

Under Section 4 of TSCA, it is the responsibility of the Agency to assure that sufficient data are developed and in a manner and quality so that hazard identification and risk assessment can be performed on chemicals identified in Section 4(a) test rules. In order to assure this, TSCA Section 4(b) specifies that each test rule must include standards for development of test data. This requirement itself is controversial in some quarters and is predicated on the fact that all chemicals cannot be completely evaluated by the same procedure (Hayes, 1975; Loomis, 1974).

After reviewing "state-of-the-art" in chronic toxicity testing, EPA agrees that total standardization of test procedures, even for studies with the same objective, is neither possible nor desirable. However, EPA believes that certain minimum standards can and must be established for certain factors of study design and conduct common to all chronic toxicity studies with the same objective. Based on historical experience, such test standards would define the minimum chronic toxicity testing requirements needed for hazard evaluations and would assure that adequate and reliable data are developed for TSCA proposes. Therefore, the standards presently proposed under Section 4(b) of TSCA would serve to standardize only those aspects of design and conduct of chronic toxicity studies which EPA finds to be essential to their scientific acceptability. Their intent is not to structure toxicological investigation so as to stifle original research or to prevent 'serendipitous' discoveries.

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[1] These estimated costs do not include the costs for prechronic toxicity studies.

Other advantages of such standards would be a better ability to compare studies and results on the same chemical or group of chemicals, a help in facilitating assessment and comparison of all studies including those from foreign countries, and a help in facilitating cost estimates (Berenblum, 1969; Page, 1976; Page 1977a).

3. Summary of Standards

An ideal test system would present a sensitive, reliable, and specific tool for detection of all possible long-term toxic effects of chemicals (J. H. Weisburger, 1976). It would also be economical, fast, foolproof, and simulate the human situation. Although presently available long-term animal studies do not fully meet these ideals (Tomatis, 1977; Shimkin, 1977), there is general agreement that such studies are the most reliable indicators of a chemical's chronic toxicity potential (D'Aguanno, 1974; Page, 1977a-b; Rall, 1977). For example, no shortened tests for oncogenicity can currently be substituted for lifetime studies when it comes to establishing the absence of oncogenic risk (WHO, 1969; WHO, 1978b).

The chronic toxicity test standards proposed for use in Section 4(a) test rules represent minimum requirements necessary to conduct an adequate study for hazard evaluation and are generally consistent with those recommended by other Federal (NAS, 1977; FIFRA, 1978; Sontag, Page, and Saffiotti, 1976; FDA, 1971; Ad Hoc Committee on the Evaluation of Low Levels of Environmental Chemical Carcinogens, 1970) and international (Canada, MHW, 1975; WHO, 1978b) agencies. They are designed to assure that TSCA test data will be developed in conformity with the following long-established criteria for chronic toxicity testing: (1) use of sensitive and reliable animal test systems; (2) optimal exposure conditions to reveal chronic toxicity; (3) elimination of extraneous factors that might influence conduct of the test and interpretation of results; (4) in-depth pathology examination to detect minute as well as more obvious adverse changes; and (5) complete documentation of all data to allow those responsible for interpretation of human relevance to make the best judgments possible (Page, 1977b).

Briefly, the main aspects of the proposed TSCA test standards are as follows:

(1) The tester must use both sexes of two mammalian species, usually rat and mouse, for the oncogenicity studies; two mammalian species, usually rat and dog, for non-oncogenic chronic toxicity studies; and three mammalian species, usually rat, mouse, and dog, for combined chronic toxicity studies;

(2) The tester must begin to dose animals as soon as possible after weaning and environmental acclimatization but no later than six (6) weeks of age for rodents and ten weeks of age for dogs;

(3) Each rodent group must contain at least 50 animals for oncogenicity studies and 58 animals for non-oncogenic and combined chronic toxicity studies; each non-rodent group must contain at least 6 animals;

(4) For oncogenicity studies, the tester must use at least three (3) dose levels (in addition to controls). The high dose level (HDL) is the maximum dose level that can be administered for the duration of the test period, with demonstrable but only slight toxicity in test aninmals, and no substantial reduction in longevity due to effects other than tumors; the second dose level is a specified level (1/4 to 1/2) of the HDL; and the third dose level is to be no more than 1/2 of the second dose level and no less than 1/10 of the HDL.

For non-oncogenic chronic toxicity studies, the tester must use at least three (3) dose levels (in addition to controls). The high dose level (HDL) must induce chronic effects including mortality; the low dose must not induce any observable evidence of adverse effects (NOEL); and the middle dose must be appropriately spaced to demonstrate a "dose-response" relationship.

For combined chronic toxicity studies the tester must use at least three (3) dose levels (in addition to controls) for the mouse and dog and at least four (4) or five (5) dose levels (in addition to controls) for the rat. The three dose levels for the mouse are those specified in the oncogenicity effects test standards and for the dog those specified in the non-oncogenic chronic effects test standards. For the rat, the high dose level is the specified HDL for the non-oncogenic chronic effects test standards; the next three dose levels are those specified for the oncogenic effects test standards; and the fifth dose level must not induce any observable evidence of adverse effects (NOEL). If one of the dose levels being specified from the oncogenic effects test standard is predicted to induce no adverse effects other than tumors, i.e., be a NOEL, a fifth dose is not required;

(5) The tester must use a matched control group identical in every respect to the exposed groups except for exposure to test substance;

(6) Route(s) of administration is (are), whenever possible, to be comparable to expected or known route(s) of human exposure;

(7) The tester must administer the test substance to mice for a minimum of 24 months but no longer than 30 months and to dogs for a minimum of 24 months. The tester must administer the test substance to rats for a minimum of 24 months but no longer than 30 months for oncogenicity studies and for a minimum of 30 months for non-oncogenic and combined chronic toxicity studies;

(8) The tester must feed test animals specified standardized diets and analyze feed and vehicle, if any, for certain specified contaminants;

(9) Appropriately trained employees must observe all animals at least every 12 hours throughout the test period;

(10) Technical employees must weigh and clinically examine each animal at least once each week during the first 13 weeks of the study and every 2 weeks thereafter. Certain quantitative clinical chemistry determinations including hematology, blood chemistry, urinalysis, function tests, and residue analysis are to be made on a minimum of eight (8) predesignated rodents in each test group and on all non-rodents. The only determinations to be made in oncogenicity studies are hematology determinations at one year and at study termination. For non-oncogenic and combined chronic toxicity studies, all of the clinical chemistry tests are to be performed at least at 3, 6, 12, 18, 24 months, and at study termination.

(11) The tester must conduct a detailed necropsy and histopathology examination of all animals with approximately thirty (30) to forty (40) tissues routinely examined microscopically;

(12) The sponsor must submit to EPA a full and detailed report of test conditions, of all observations made on test animals, and of any data analysis conducted;

(13) The tester must conduct all studies according to specified good laboratory practice standards.

Exact and complete details of these test standards are to be found in the proposed test standards themselves.

In the discussions to follow, the Agency outlines the scientific or other basis for each of the standards proposed and discusses the major issues associated with the development and use of these standards.

B. DESIGN ISSUES

1. Introduction

A study to determine toxic effects of a chemical consists of three elements--study design, study conduct, and data analysis. Each plays an essential role in the final outcome and usefulness of long-term animal studies. A properly designed study ensures that an adequate data base is generated during the study to meet its objectives; a properly conducted study ensures the quality of the data base generated, and a properly analyzed study enables a decision to be made as to chronic toxicity potential of the test substance under the given test conditions. The following is a discussion of aspects involved in proper design of a chronic toxicity study.

2. Test Species, Strain, and Sex

Objective: The standards for selection of test species, strain and sex are set forth to ensure that sensitive and reliable animal test systems are selected.

a. Test Species

i. Oncogenicity Studies. Testing is required in at least two mammalian species, usually the rat and the mouse. Present knowledge indicates that development of chemically induced neoplasia is the result of a complex series of biological interactions which are subject to and controlled by numerous endogenous and exogenous modifying factors (Hueper and Conway, 1964; J.

H. Weisburger, 1973). Because of this complex pathogenesis, no one species of animal can be predicted to give biological responses similar to those of humans. As discussed in detail below, certain scientific, technical, and economical considerations, thus, become the deciding factors in selecting appropriate test animals. Included among these factors are the life span of the species, susceptibility to tumor induction by chemical oncogens, reliability as a model for the human, quality of the animals in terms of health and stamina, physical size, genetic stability and reproducibility, availability in sufficient number, purchase cost, maintenance costs, knowledge of spontaneous tumor incidence, and availability of historical information (FDA, 1959; NAS, 1961; Clayson, 1962; Weisburger and Weisburger, 1967; Canada, MHW, 1975; Page, 1977a; WHO, 1978b; FSC, 1978).

One of the most important considerations in selecting animals species for evaluation of potential chemical oncogens is the normal life span of the animals. Because tumors usually develop only after a long latent period, (e.g., humans 5 to 40 years), it is usually necessary to extend the period of testing over the greater part of or the entire life span of the animals (Zwickey and Davis, 1959; NAS, 1961; Magee, 1970; NAS, 1975; Canada, MHW, 1975; Page, 1977b; WHO, 1978b; FSC, 1978). This latency period depends on the life span of the animal and the potency of the chemical. Even with potent chemical oncogens, it takes a minimum of 1/8 to 1/4 of the life span of a given species for chemically induced tumors to develop (Weisburger and Weisburger, 1967; D'Aguanno, 1974). For this reason, species with long life spans such as the rabbit (7 years), dog (> 10 years) and monkey (> 10 years) are generally not used for routine oncogenicity testing (Arcos, Argus, and Wolf, 1968). Comparing the latent period for induction of neoplasms with benzo(a)pyrene in a range of animal species, it was noted that while the rat, mouse, guinea pig, and rabbit all responded within two years, it required up to ten years to develop in monkeys (Hartwell, 1951; E.K. Weisburger, 1971; Canada, MHW, 1975). Although the dog is relatively unique in that it shows the same organ specificity to aromatic amine carcinogenesis as the human, it is not uncommon for the latent period to be five to ten years in these animals (Canada, MHW, 1975; J.H. Weisburger, 1975; Stula, Barnes, et al., 1978a-b).

Susceptibility of species to particular chemical oncogens, classes of chemical oncogens, or routes of exposure must also be considered in selection of appropriate test species (Hueper and Conway, 1964; Shimkin, 1974; NAS, 1975; J. H. Weisburger, 1976; Sontag, 1977). Species specificity reflects the definite and sometimes decisive role which the host organism plays in controlling the response to oncogenic exposure (Hueper and Conway, 1964; J. H. Weisburger, 1976). For example, guinea pigs show an apparent resistance to a wide variety of chemical oncogens (Page, 1977b). Most strains of rats are resistant to induction of tumors by 2-naphthylamine, a potent oncogen in other species including humans (J. H. Weisburger, 1973). Rat skin is relatively resistant to hydrocarbon oncogens while mouse skin is susceptible; therefore, the mouse is the species of choice when testing this class of chemicals (Clayson, 1962).

Not only must a species be sensitive to tumor induction by chemical oncogens, but it must also be a reliable model for the human. If extrapolation of data to humans is to be meaningful, a species must have a low potential for generating both false positive and false negative results. Of the 26 chemicals known to be oncogenic in humans, all but three, arsenic, benzene, and phenacetin-containing analgesics, have induced tumors in some animal model; the majority showing effects in rat and mouse (Clayson, 1978b; Tomatis, Agthe, et al., 1978). Of 56 other chemicals suspected of being oncogenic in humans, all have tested positive in animal studies (Maugh, 1978). While it is possible to estimate the false negative rate (3/26) for long-term animal studies, it is not possible to estimate a false positive rate because of the complexity of determining which chemical oncogens are active in humans (see discussion in Section I).

Other factors also influence the choice of species to be used. Since test animals must be maintained for the greater part of or for their entire life span, it is important that the animals be of high quality in regard to their health, vigor, and stamina. Ideally, spontaneous disease rates should not exceed 5 percent (FSC, 1978). Because proper evaluation of data requires that large numbers of animals be used (see Section B.4.), appropriate species should be small in size for maintenance and cost purposes, be available in sufficient numbers, be genetically stable and readily reproduce. Proper evaluation of data also dictates that the species have a low spontaneous tumor rate with these tumors appearing as late in life as possible. Information regarding the susceptibility to chemically induced tumors, the type and incidence of spontaneous diseases, and the metabolism and pharmacokinetics of the test substance also aids in the selection of appropriate species.

At this time, it is generally agreed that the only mammalian species that adequately meet these selection criteria are rodents, especially the rat and mouse (Barnes and Denz, 1954; FDA, 1959; WHO, 1961; Berenblum, 1969; Magee, 1970; Peck, 1974; NAS, 1975; Canada, MHW, 1975; Sontag, Page and Saffiotti, 1976; J.H. Weisburger, 1976; NAS, 1977; Sontag, 1977; WHO, 1978b). These two species have a relatively short life span, are susceptible to chemically induced oncogenesis and are small in size, readily available, relatively cheap, fertile, hardy, and well-standardized and studied.

Since it is not presently possible to predict which species will give oncogenic responses similar to those of humans and since there are many examples of species variation to oncogens on record among rodents, it is considered necessary to test a chemical in more than one species (Shubik and Sice, 1956; FDA, 1959; Della Porta, 1963; Berenblum, 1969; FDA, 1971; NAS, 1975; Page, 1977b, Sontag, 1977; WHO, 1978b; FSC, 1978). The more species a chemical is tested in, the less chance there is of missing an oncogenic response due to metabolic or other variables (Sontag, 1977). Due to economic, space, and time considerations, however, more than two species are rarely used[1/]. Testing in one rodent species costs approximately $200,000 and requires 4-5 years to complete.

Testing in more than one species not only increases the chance of detecting an oncogenic response but also increases the confidence in extrapolating to predict carcinogenic potential to humans. Although a positive oncogenic effect in one species is considered adequate evidence of a potential hazard to humans, positive results in more than one species verify the potential hazard and give an indication of the relative potency of the chemical in different species. Although no unqualified negative answer is ever possible, a lack of evidence of tumor induction in at least two species is generally regarded as the most reliable criterion of non-oncogenicity (WHO, 1969; WHO, 1978b). This is very important since epidemiological data from humans is never adequate to define a negative oncogen (Clayson, 1978a).

1/ Testing in only two species, of course, is not foolproof as demonstrated by the inability to induce tumors in animals with arsenic, known to be oncogenic to humans (Bencko, 1977).

ii. Non-oncogenic Chronic Toxicity Studies. Testing is required in at least two mammalian species, one the rat and the second a non-rodent, usually the dog.

(A) Need for Two Species

The requirement for two species in non-oncogenic chronic toxicity testing is due to the uncertainties of extrapolating to humans from a single surrogate for such effects (Barnes and Denz, 1954; Benitz, 1970; Loomis, 1974; Hayes, 1975). As in the case of oncogenicity, significant differences in susceptibility and mode of action for non-oncogenic chronic toxicity exist between species and have been identified as due to differences in distribution, metabolism, and cytology (Albert, 1973). McConnel, Moore, et al. (1978) reported a positive correlation between liver burden of TCDD in mice, rats, guinea pigs, and monkeys and the degree of toxicity induced in these species. Williams (1978) describes numerous species variations in detoxification mechanisms. Hayes (1975) cites species differences in toxicity to norbormide of 230 fold and to thalidomide of 1,000 fold. Hodge, Smith, et al. (1963) reports a 250 fold difference in sensitivity to fluoroacetate among mammals and if the toad is included, a 25,000 fold difference. In addition, cause of death from fluoroacetate differs between species; the dog displays central nervous system effects, the rabbit shows cardiac effects, and man and monkey show a mixed response. Casida and Baron (1976) cite marked species differences in susceptibility and clinical signs of neurotoxicity due to organophosphorus compounds. In this case, humans and mature hens appear to respond similarly; the dog, cat, pig, cow, sheep, and horse, though susceptible, show different patterns of toxicity; and the rat appears to be refractory. These differences in species susceptibility demonstrate the weaknesses inherent to relying on only one animal model for extrapolation to humans for non-oncogenic chronic toxicity effects.

(B) Need for a Rodent and a Non-Rodent

Among laboratory rodents, mice and rats remain the species of choice under most general circumstances for chronic toxicity studies (FSC, 1978). As discussed in the section on selection of species for oncogenicity testing, the life span, small size, ready availability, cost, fertility and hardiness of rats and mice and the fact that they are well-standardized and studied make them suitable for chronic toxicity testing. Of these two species, the rat was chosen as the species of general choice for the non-oncogenic chronic toxicity studies because of the enhanced capability to conduct clinical evaluations with larger-sized animals and because of the more extensive work done with this species. It is felt that blood collection in the mouse is too stressful and that blood volume is too limited.

Nephroses, chronic pulmonary disease, hypertension and deposition of lipids in the aorta, susceptibility to infections, senility as reflected by morphological changes in the CNS associated with aging, growth, and survival in terms of the median life span have been described in laboratory rats. Although differences in long-term pathogenesis between man and rat have been identified (e.g., arteriosclerosis) the qualitative similarities are striking.

While the use of a rodent in chronic toxicity testing is non-controversial, the use of a non-rodent is highly controversial. Those challenging the need for a non-rodent contend that nearly all chronic effects will be observed in the rodent (Aviado, 1978). Examination of the available data, however, shows important differences. For example, the report by Vettorazzi (1975) shows differences in response between the rat and dog in two year feeding studies with binaparcyl, amitrole, dichlofluanid, thiabendazol, and dodine. Hodge, Downs, et al. (1968) found similarities in the toxicities of monuron and diuron while linuron showed species differences between rat and dog. This latter example shows that even where similarities exist between species for one chemical differences may exist for related substances.

EPA has decided to require use of a non-rodent in chronic toxicity studies for three reasons; First, EPA believes it is necessary for Section 4(a) purposes to utilize as efficient a test system as practical to detect all possible chronic effects. Since nononcogenic chronic effects are sometimes detectable in non-rodents but not in rodents (or vice versa), it is felt that both types of species must be used. Second, use of the non-rodent will enhance the capability to conduct more precise clinical evaluations. Third, since the Agency will likely propose exposure limits for certain chemicals tested under Section 4 where positive test results are found, dose-response data with two species will provide a firmer scientific basis for risk estimations. Generally, dose-related effects obtained with larger animals more nearly approximate those expected in humans. For example, Litchfield (1961) in an analysis of 39 signs of toxicity of six drugs in the rat, dog and human found the dog to be a better predictor of human effects than the rat.

Dogs have been extensively used as the usual non-rodent species of intermediate life span between humans and the common laboratory rodents. They are of a convenient size and disposition for clinical examinations, and can be obtained as purebreds if genetic similarity is desired. The cost of using dogs ($226,400) instead of another rodent species ($257,300) is insignificant.[1/] Because of the important differences in information obtained from using both a rat and dog and because cost of such studies are equivalent, EPA believes that a two year dog study is feasible and necessary to adequately determine chronic toxicity.

Non-human primates have also been promoted as surrogates for humans based primarily upon their phylogenetic relationship and similarity in physiological functions. However, they are generally difficult and expensive to obtain in a defined (disease-free) condition with most animal sources from foreign countries having restricted exportation quotas. For routine testing, the advantages of non-human primates over dogs do not offset the procurement, cost, and management problems involved in their use.

iii. Combined Chronic Toxicity Studies. Testing is required in at least three mammalian species, two rodents, usually the rat and mouse, and a non-rodent, usually the dog.

As discussed in detail in the previous two sections, at least two species, usually the mouse and rat, must be used to adequately test a chemical for potential oncogenic effects and two species, usually the rat and dog, must be used to adequately test a chemical for potential non-oncogenic chronic effects. Based on these assessments, EPA believes that to adequately assess both oncogenic and non-oncogenic chronic effects in one test all three species must be used. The mouse is used to detect oncogenic effects, the dog non-oncogenic chronic effects, and the rat both types of effects. Use of the dog to detect oncogenic effects is not appropriate because the duration of the study is too short for tumor development in a long-lived species such

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[1/] For cost estimates see section III.

as the dog except for the most potent of oncogens.

(iv) Alternative Species. The Agency recognizes that the mouse, rat and dog are not always the most appropriate species in which to test certain chemicals. Because of this, EPA allows use of alternative species. The main objective of the test standards is not only to show toxic effects but to obtain the most meaningful data for extrapolation to the human situation. Any procedure which allows this to be accomplished will be accepted by EPA if an appropriate rationale can be shown to support it . Acceptable rationale for use of another species would be to show that is a better model for a given chemical because of its known sensitivity to a given class of chemical toxicants or because of its known metabolic or pharmacokinetic handling of the chemical.

b. Test Strains

No concensus exists in the scientific community as to the most appropriate type of strain to use, outbred, inbred or hybrid (NCTR/NCI, 1979). Because of this, EPA has decided not to require use of either type but leaves the decision to the tester.

Outbred stocks are animals that are maintained by a breeding system that tends to minimize inbreeding and, thus, maximize genetic variation (ILAR, 1976). Inbred strains are animals that have been brother-sister mated for 20 generations or its equivalent (Clayson, 1962; NAS, 1974). Genetic variation within an inbred strain is minimal. Hybrids are animals resulting from a cross between two inbred strains (ILAR, 1976). Use of any one of the three types of strains has its advantages and disadvantages.

Arguments over use of inbred or hybrid strains versus outbred stock characterize the two possible approaches to long-term testing, namely to simulate the human situation or to maximize sensitivity of the test model (Tomatis, 1977). Certain researchers and scientific committees recommend use of outbred stock because, like humans, they are genetically heterogenous (Della Porta, 1963; Arcos, Argus, and Wolf, 1968; FDA, 1971; NAS, 1975; Canada, MHW, 1975). Because of their heterogeneity, it is believed that at least a few animals will respond if a chemical has activity and that extrapolation of the data to humans is more meaningful. In reality, compared to humans, outbred strains have narrow genetic bases because each colony has a restricted and self-limiting gene pool (Sontag, 1977). Compared to inbred strains, the outbred stocks have a wider genetic base and generally longer life spans, and are more resistant to infections (Sontag, 1977; FSC, 1978). The major disadvantage with using outbred stocks is their fluctuating incidence of spontaneous diseases including neoplastic lesions and variable end-response. Because of this, larger numbers of animals than would be the case for inbreds are needed to secure statistically significant results (Weisburger and Weisburger, 1967).

Other researchers and scientific committees recommend use of inbred or hybrid strains because of their genetic homogeneity (Zwickey and Davis, 1959; Weisburger and Weisburger, 1967; Berenblum, 1969; Festing, 1975; Sontag, Page and Saffiotti, 1976; FSC, 1978). This genetic stability of inbred and hybrid strains allows for greater uniformity of response, highly predictive spontaneous disease and tumor incidences and better reproducibility of test results. However, their homogeneity may also be their major disadvantage in that they are more likely to be totally resistant to certain potentially toxic chemicals than are outbred animals (Clayson, 1962; Sontag, 1977). Hybrids have the additional advantage over inbred strains in that they are more vigorous, less disease prone and have a longer life expectancy. Their disadvantage is that they are more difficult to obtain and, therefore, tend to be more expensive.

c. Sex

Because of known sex differences in response and possible target organs, the tester must use both sexes to show the full range of activity of a test substance. Sex differences in response to chemical toxicants are well documented in both humans (American Cancer Society, 1978) and test animals (Bock, 1964; J.

H. Weisburger, 1975; Canada, MHW, 1975). Sex-linked responses may involve differences in incidence of effects, organ specificity, and latent period. For example, N-2-fluorenylacetamide induces liver cancer primarily in male rats (Miller, 1970) while 7, 12-dimethylbenz(a)anthracene induces a higher incidence and multiplicity of skin tumors in male mice than in female mice (E. K. Weisburger, 1971). Dichlofluanid damages the testes in dogs (Vettorazzi, 1975). Hodge, Downs, *et al*. (1968) found an abnormal blood pigment in female dogs at 25 ppm while at 625 ppm the abnormal blood pigment was present in both sexes.

3. Age at Start of Study

Objective: The standards for age at start of a study are designed to maximize the sensitivity of the animal test system and to allow sufficient time for toxic effects to develop.

a. Weanlings. According to the proposed test standards, the tester must begin to dose animals as soon as possible after their weaning and environmental acclimatization but no later than six (6) weeks of age for rodents and ten (10) weeks of age for dogs.

There is general agreement that animals must be started on treatment at a young age in order to maximize the sensitivity of the animal bioassay system (NAS, 1961; Weisburger and Weisburger, 1967; Arcos, Argus, and Wolf, 1968; WHO, 1969; Berenblum, 1969; Magee, 1970; Tomatis, 1974; NAS, 1975, Canada, MHW, 1975; Sontag, Page, and Saffiotti, 1976; Weisburger, 1976; WHO, 1978b; FSC, 1978). However, a great deal of discussion and some controversy has arisen over the past several years over the use of prenatal and neonatal animals versus use of weanling animals. (FIFRA, 1978; Rice, 1976; Toth, 1968). EPA requires the use of weanling animals in general oncogenicity testing. This is because weanling animals actively undergoing protein synthesis and cellular proliferation/maturation, and subject to alterations in physiology occurring during sexual maturation usually are more responsive to chemical toxicants including chemical oncogens than adult animals (Weisburger and Weisburger, 1976). Use of weanling animals also allows the test substance to be administered for the major portion of the animals' life span, allowing for extensive exposure and maximum time for development of toxic effects.

b. *In utero*. Theoretically, in order to detect all age-related effects, animals should be exposed during all phases of their lives, including during gestation (Canada, MHW, 1975; Tomatis, 1974).

However, after reviewing the available data, EPA concluded that the scientific basis coupled with certain technical problems is such that prenatal and neonatal animals should not be required for general oncogenicity testing instead of weanlings.

In utero testing for potential chemical oncogens took on new importance when diethylstilbestrol (DES) was shown to be oncogenic to humans following prenatal exposure (Herbst, Ulfelder, and Poskanzer, 1971a-b). Tumors in offspring of exposed mothers have now been shown to be induced by at least 30 chemicals (Tomatis, 1974; Rice, 1976). However, all of these chemicals were already known to produce tumors in adult and/or newborn animals when they were tested (Tomatis, 1974; Rice, 1976). Because too few chemicals not oncogenic in adults have been tested via *in utero* exposure, the extent of enhanced sensitivity of this method is difficult to quantify. Fetal tissue may be more susceptible or resistant to certain chemical oncogens simply due to their lack of metabolic competence or to changes in sensitivity of tissues at different stages of development (Rice, 1976). EPA believes more work needs to be done to validate this test method before it can be adopted as a testing requirement.

EPA is also hesitant to require *in utero* testing, because of various technical difficulties. Restricting administration of the test substance to the period of gestation may result in inadequate exposure and thereby negate use of any negative results. Use of this method does not decrease the length of time of the study, especially in regard to negative results. The amount of chemical administered is very difficult to predict because of possible fetal toxicity including teratogenicity. To achieve use of maximum tolerated doses, it is usually necessary to adjust levels for various ages, thus making dose-response relationships difficult to evaluate.

c. Neonatal: Following the pioneer work of Pietra, Spencer and Shubik (1959), it was thought that using neonatal animals might allow for reducing the number of test animals and length of exposure for oncogenicity and chronic toxicity studies (Della Porta, 1963). Studies of drugs and pesticides also indicated that newborn animals were generally more sensitive than adults with a mean ratio of response to a given dose (newborn/adult) of 2.9 and a range of 0.6-10.0 (Hayes, 1975). Careful review of available data indicates that neonatal treatment alone cannot be recommended in some instances since certain known oncogens have gone undetected in this method (Della Porta and Terracini, 1969). EPA believes that more work needs to be done also to validate this test method. Use of neonatal animals also entails many of the same technical problems as use of prenatal animals, i.e., inadequate exposure and differences in metabolic competence and tissue sensitivity.

4. Number of Animals/Test Group

Objective. The standards for selection of numbers of animals per test group are set forth to provide for sufficient number of test animals per group so that reliable statistical analysis can be used to evaluate the validity of test results.

a. Number. Each group of rodents must contain at least 50 animals for oncogenicity studies and 58 animals for non-oncogenic and combined chronic toxicity studies. The eight additional animals required for the non-oncogenic and combined chronic toxicity studies are the eight predesignated animals need for clinical chemistry studies. Each group of dogs must contain at least 6 animals.

Ideally, in arriving at these numbers, EPA would consider the desired sensitivity of the test, expected incidences of comparable spontaneous diseases or tumors in control animals, and percentage of animals that are expected to survive to an adequate age to show chronic effects (referred to as the effective number of animals) (Page, 1977a). However, as discussed below, the final numbers of animals required reflect a compromise between the optimal sensitivity and practical factors such, as costs and availability of animals (in case of dogs or primates).

The number of animals in each test group is an integral part of the sensitivity (i.e., the ability of the study to demonstrate the effect(s) studied and the reliability of the observed effect(s)) required for statistical analysis of test data (Barnes and Denz, 1954; Shubik and Sice, 1956; Vos, 1959; NAS, 1961; Arcos, Argus, and Wolf, 1968; Berenblum, 1969; Magee, 1970; NAS, 1977; Page, 1977b; FSC, 1978). Sensitivity of a test depends on the smallest difference in disease or tumor incidences between exposed and control groups the study is designed to detect and the degree of confidence with which this difference should be detected (Clayson, 1962; Arcos, Argus, and Wolf, 1968). Type of data is also a consideration; data may be qualitative, i.e., presence or absence of effects, or quantitative, i.e., latent period or time to effect.

In statistical significance testing the minimal detectable difference, (delta), between exposed and control groups is dependent upon the standard error of the mean. The standard error is defined as ($\sigma/\sqrt{n}$) where (σ) refers to the standard deviation of the distribution and n refers to sample size. Because the standard error term is used as the denominator in the minimal detectable difference term, the smaller the difference to be detected, the larger the sample size requirements in the protocol.

In chronic toxicity testing, sensitivity also involves minimizing the probability of false positives and false negatives. A false positive occurs when an effect is judged to be the result of exposure to the chemical (e.g. oncogenicity based upon the test results) when the results are actually spurious or not the result of exposure to the test substance. A false negative is defined as failure to correctly classify or detect the toxic effect. The risk or probability which a tester allows for false positives is referred to as Type I error (alpha); 1 minus the probability of alpha is denoted as the level of statistical confidence (i.e., the ability to detect a true positive). Probability of not detecting a toxic effect is referred to as Type II error (beta); 1 minus the probability of beta is denoted as statistical power (i.e., the ability to detect a true negative) (Fears, Tarone, and Chu, 1977; Fleiss, 1973). In determining the level of confidence and power in study design, a statistical distribution type for occurrence of Type I and II errors is assumed, such as the lognormal, Poisson or binomial.

Because an unlimited number of animals can not be tested each time, a limitation on sensitivity must be set for general testing of unknown chemicals for toxicity potential. Results of biological investigations generally have been considered to be significant if the probability (p) that the difference in incidences of diseases or tumors between control and exposed groups due to chance is relatively low, i.e., p=0.05 (NAS, 1961). This "p" value is referred to as the significance level, or probability of false positives. As illustrated in Table 1 for p=0.05, the decision as to whether results produced in an exposed group by the test substance is different from the result in the control group is dependent on the incidence of comparable spontaneous diseases or tumors in control animals and on the effective number of exposed animals (Page, 1977b).

Table 1

Incidence of Tumors in Exposed Groups Required for Significance (p=0.05) Depending on Test Group Size and Incidence of Tumors in Controls*

Incidence of Tumors in Controls (%)	No. of Animals Per Group** 10	25	50	75	100
0	50%	20%	12%	8%	6%
10	70	40	28	24	21
20	80	52	40	36	34
30	90	64	52	47	45
40	100	72	62	58	55

*Calculations based upon tabulations of Mainland and Murray (1952) and presented by Page (1977a).

**Exposed and control groups of same size.

In practice, one usually cannot predict in advance how many test animals will survive to an adequate age to show effects or whether effects that may be induced will represent a generalized elevation of the type which arise spontaneously in controls or will represent a type practically nonexistent in controls. For these reasons, one cannot specify error rates (e.g., 95% probability of detecting a 10% increase in tumor incidence between exposed and control groups) to determine sample size for each specific study. Because one cannot specify error rates, historically, a minimum of 50 rodents or 4-8 non-rodents per test group has been selected (Goldenthal and D'Aguanno, 1959; Benitz, 1970; Newberne, 1975; NAS, 1975; Sontag, Page and Saffiotti, 1976; Page, 1976; Page, 1977a-b; FIFRA, 1978). This represents a compromise between the desired sensitivity of the test system and practical considerations, such as cost and quantity of work. As discussed previously, for biological investigations the significance level is usually set at p=0.05 (i.e., there is no greater chance of being wrong than once in 20 such studies). The economic factor is easily seen by the cost of the non-oncogenic chronic health effects test which requires 50 rats per group and 6 dogs per group and is estimated to cost $550,000 (see Section III). Another factor that must also be considered is quanitity of work. EPA agrees with Benitz's basic concept that more useful information can be obtained in thorough studies carried out in a relatively small number of animals than in incomplete studies using an excessive number of animals (Benitz, 1970).

EPA also agrees with Benitz that clinical chemistry testing cannot be done on all 50 rodents and that 8 animals per group will give sufficient information for Section 4 needs (Benitz, 1970). These 8 animals are in addition to the normal 50 rodents per group because the manipulation of the animals during the clinical chemistry tests may alter the effect of the test substance.

b. Randomization: Another important element in statistical analysis of test data is proper allocation of animals to exposed and control groups (Arcos, Argus, and Wolf, 1968; Berenblum, 1969; Roe and Tucker, 1974; Peck, 1974; Sontag, Page, and Saffiotti, 1976; NAS, 1977; Page, 1977b; FSC, 1978). Randomization of animals is necessary to ensure that unintentional selection biases are not introduced into the study. The test standards do not specify use of a given randomization method. NCI recommends that all animals be from the same supply source, ideally within 2-3 days of the same age, and optimally assigned to test groups and cages by the use of a table of random number (Sontag, Page, and Saffiotti, 1976). Other methods of randomization are available such as Weiner's complete block design (Weiner, 1972).

5. Number of Dose Levels and Dose Selection

Objective: The standards for selection of dose levels are designed to optimize exposure conditions to reveal any toxic response, to ensure that adequate data will be available at the end of the study, and to obtain information, if possible, on dose-response relationships.

a. Oncogenicity Test Standards. The range of dose levels selected for oncogenicity testing must include one which is the maximum that can be administered for the duration of the test period, with induction of demonstrable but only slight toxicity, and no substantial reduction in longevity due to effects other than tumors (HDL); one which is a specified level

(1/4 to 1/2) of the HDL, and one which is less than 1/2 of second dose but no less than 1/10 of the HDL.

i. High Dose Level--Need: The main controversy in selecting dose levels for oncogenicity tests involves the use of an upper dose level that is in the toxic range (Page, 1977b; Munro, 1977). Use of such a high dose level is intended to achieve maximum sensitivity of the test system without altering its accuracy (NAS, 1961; Weisburger and Weisburger, 1967; WHO, 1969; Friedman, 1974; Sontag, Page, and Saffiotti, 1976; WHO, 1978b; FSC, 1978). As discussed in the previous section, the number of animals (50 per group) tested is, of necessity, extremely small compared to the size of the human population potentially at risk. A chemical which induces tumors in one percent of the U.S. population would result in over two million new cancer cases. Yet an exposed group of 50 animals must have at least a twelve percent incidence of tumors (if controls have a zero percent incidence) for results to be considered statistically significant at the p=0.05 level (Table 1). It is believed that a chemical oncogen, which at environmental or occupational levels is a threat to humans, will cause a statistically significant detectable increase in tumor incidence when administered at much higher dose levels to a small population of test animals. Within the limitation of the animal's ability to tolerate the chemical for long-term exposure without death or substantial life-shortening effects, usually the higher the dose is the higher the tumor incidence and the shorter the latency period (J.H. Weisburger, 1976). Based on this data, EPA believes that it is necessary to use a dose level in the toxic range to optimize revealing potential oncogens.

ii. High Dose Level--Definition: Proper selection of the HDL is difficult because there is no general agreement as to what parameters should be used to define this dose level (Sontag, Page, and Saffiotti, 1976; Munro, 1977; Page, 1977b). To achieve maximum sensitivity within a test system, it has generally been recommended or required that a maximally tolerated dose be used as the high dose level (Sontag, Page, and Saffiotti, 1976; NAS, 1977; WHO, 1978b). This has been defined most accurately by NCI to be the highest dose that causes no more than ten percent weight decrement as compared to appropriate control group(s) and is predicted to produce no lesions (other than those related to a neoplastic response) that would be expected to shorten the animal's natural life span (Sontag, Page, and Saffiotti, 1976). The proposed tests standards require a HDL which is the maximum dose level that can be administered for the duration of the test period, with demonstrable but only slight toxicity in test animals, and no substantial reduction in longevity due to effects other than tumors. EPA has defined its required upper dose level slightly different than NCI because it wants to make sure that this level is slightly toxic but without an effect on longevity. Weight reduction is only one acceptable demonstrable parameter of slight toxicity.

Major criticisms of using a toxic dose, whether defined as a MTD or HDL, are: (1) at high levels, normal metabolic pathways may become saturated leading to aberrations in metabolic pathways which are predisposing to tumor development; (2) this dose level may be incompatible with normal physiological function; and (3) this dose level may be unrealistic compared to human exposure (Munro, 1977). A ten percent decrement in body weight is not considered normal physiology. EPA recognizes such problems but agrees with the present general scientific opinion that if the high dose group shows no sign of chronic toxicity, decrease in mean weight, the oncogenic potential to the chemical substance may be underestimated, i.e., the study will have a higher probability of being a false negative (J.H. Weisburger, 1976; Sontag, Page and Saffiotti, 1976; Page, 1977b; WHO, 1978; FSC, 1978). Oncogenicity is a dose dependent effect with some chemical oncogens having fairly steep dose-response curves (Weisburger and Weisburger, 1967). For these chemicals, the tumor yield falls off rather rapidly with relatively minor alterations in dose level. By not employing highest possible doses, the effect might go undetected.

The term "maximally tolerated dose" (MTD) has almost as many different connotations as there are individuals who use it and is, therefore, subject to misinterpretation. In practice, predicted MTD's have often not been achieved such that toxic effects considered "not tolerated" occurred. Most importantly because EPA is requiring use of a slightly toxic dose, and to avoid confusion in use of MTD, EPA prefers to use the term HDL. It is believed that this will allow the emphasis to be placed on the important biological questions that need to be addressed.

iii. High Dose-Level--Determination: To predict the HDL most accurately involves undertaking of expensive, time-consuming pharmacokinetic and metabolic studies. With these types of information, it is possible to predict accumulation in the body and dose-dependent changes in the metabolic profile. In practice, however, to select the HDL, it is necessary to use an empirical approach based on results of a subchronic toxicity study (Sontag, Page, and Saffotti, 1976). Review of data from the NCI bioassay program (Page, 1977b) shows that considerable variability exists in being able to assess the length of the subchronic toxicity study needed to accurately predict long-term toxicity. Clearly, the use of 4-6 weeks subchronic studies is not satisfactory (Burchfield, Storrs and Kraybill, 1974). Indeed, for some chemical substances, a toxicity study of 20-30 weeks or longer may be needed to provide an accurate estimate of two-year toxicity. For most however, reasonable estimates can be made on the basis of a 90 day study. After reviewing available data and cost figures, EPA believes that routine subchronic toxicity studies of at least ninety-days are necessary to properly predict long-term toxicity in oncogenicity studies.

iv. Second and Third Doses: In theory, only one dose level is needed to detect oncogenic potential of a chemical. But, because of problems in being able to accurately predict this HDL, and because under TSCA a risk assessment must be conducted once a chemical oncogen has been identified, EPA is requiring the use of at least three dose levels. Use of three dose levels is in agreement with recommendations of other major scientific bodies (Canada, MHW, 1975; NAS, 1977; FIFRA, 1978; WHO, 1978; FSC, 1978).

A second dose level acts as a safety factor if the HDL is overestimated and survival rate or toxicity level is such that results from this group cannot be used. A second dose level is also needed because the HDL may not produce maximum tumor incidence because of competing cytotoxic and oncogenic activities (Arcos, Argus and Wolf, 1968).

If results from all three dose levels can be used, use of the third dose level provides for better knowledge of dose-response relationships, and, therefore, for better precision at estimating human health risks (NAS, 1961; Page, 1977b). Use of at least three dose levels allows for concurrent data collection for both determining oncogenic potential and doing curvilinear risk assessment. This is both cost and time effective in that a second study will not have to be done to obtain this information. The third dose level also acts as a safety factor in case results from the HDL cannot be used or in the extreme case where results from both HDL and the second dose level cannot be used.

Ranges are set for the second (1/4-1/2 HDL) and third (1/2 second dose - 1/10 HDL) dose levels for two reasons: (1) to achieve the purpose(s) for using each of the dose levels as discussed in the previous two paragraphs; and (2) to allow for variations in dose-response. The range for the second dose level is in agreement with that recommended by NCI (Sontag, Page and Saffiotti, 1976) and FIFRA (1978). The third dose level is the same as that recommended by the FIFRA guidelines except for setting a lower limit of 1/10 HDL. This lower limit was set because of the limited sensitivity of the study. With the use of only 50 animals per group, it is anticipated that the sensitivity of the study will be too low to detect activity below this level for most oncogens. This dose level is not meant to be a threshold or NOEL value for oncogens.

b. Non-oncogenic Chronic Toxicity Test Standards.

A minimum of three dose levels are required for non-oncogenic chronic toxicity studies in order to meet the objectives of such tests. The objectives are to detect chronic effects and to establish dose-response patterns and "no-observed-adverse-effect levels. Therefore, the range of dose levels selected for chronic toxicity must include one eliciting a clearly toxic response, one showing no-observable effects (NOEL), and at least one intermediate level showing a certain level of toxicity. The intermediate dose level(s) is required to be selected so as to maximize information on dose-response relationships and to allow application of risk-analysis models.

As in oncogenicity studies, to predict the high and low dose levels most accurately involves the undertaking of expensive, time-consuming pharmacokinetic and metabolic studies. With these types of information, it is possible to predict accumulation in the body and dose-dependent changes in effects. In practice, however, to select the appropriate high and low dose levels, it is necessary to use an empirical approach based on results of subchronic toxicity study. As stated previously, review of data from the NCI bioassay program (Page, 1977b) shows that considerable variability exists in being able to predict long-term toxicity. After reviewing available data, EPA believes that routine subchronic toxicity studies of ninety-days duration are necessary to properly predict long-term toxicity.

c. Combined Toxicity Test Standards.

For combined toxicity studies the tester must use at least three (3) dose levels (in addition to controls) for the mouse and dog. The three dose levels for the mouse are those specified in the oncogenicity effects test standards and for the dog those specified in the non-oncogenic chronic effects test standards. The reason for this is that the data from the mice studies will be used for determining oncogenicity potential while the data from the dog studies will be used only for determining non-oncogenic chronic toxicity potential.

The requirements for rats in the combined effects studies are different from the other two species because data from such studies will be used to determine both oncogenic and non-oncogenic chronic toxicity potential. In order to accomplish this purpose, enough dose levels must be used to insure that proper and sufficient information is collected on both effects. As discussed in the previous section, one of the dose levels selected for non-oncogenic chronic toxicity testing must elicit a clearly toxic response, one must show no-observable effects (NOEL) and one is to be an intermediate level showing a certain level of toxicity. For the oncogenic toxicity testing, one dose level is to elicit slight toxicity with the other two being some specified fraction of the higher slightly toxic dose.

EPA believes these two tests can be combined if four or five dose levels are used to provide adequate information to study both effects. One dose must clearly elicit a toxic response to fulfill the objectives of the upper dose for the non-oncogenic chronic toxicity studies; a second dose must elicit only slight toxicity as defined in the oncogenicity effects standards to fulfill the objectives of the high dose level for the oncogenicity studies; the third and fourth doses must be the lower two doses defined in the oncogenic studies in order to adequately assess for oncogenicity potential; the fifth dose must be a no-observable effect level to fulfill the second objective of the non-oncogenic chronic effects test standards. The requirements for an intermediate dose level to show dose response relationship(s) for non-oncogenic chronic effects can be fulfilled by the second dose level described above. The requirements of the fifth dose level can be fulfilled if either the third or fourth dose levels are predicted to induce no adverse effects other than tumors. Use of these four or five dose levels in the rat should enable one to determine both the oncogenicity and chronic toxicity potential of a chemical and a NOEL for effects other than oncogenicity.

6. Controls

Objectives. The standards for use of control groups are designed to assess possible contribution(s) made by any factor(s) other than the test substance itself and to determine normal survival rates and spontaneous disease and tumor incidences, factors which are necessary for proper interpretation of test results.

a. Matched Controls. The tester must use a matched control group identical in every respect to the exposed groups except for exposure to the test substance. This will be a vehicle control group if a vehicle is used to administer the test substance or a negative (untreated) control group if no vehicle is used.

Study of matched controls is necessary for two reasons. First, as in most toxicity studies, it is necessary to assess the possible contribution made by any factor(s) other than the test substance itself. Second, and extremely crucial for interpretation of results from chronic toxicity studies, is the need to determine the normal life span and extent of natural diseases including spontaneous tumors in comparable control animals (Arcos, Argus and Wolf, 1968; WHO, 1969; Roe and Tucker, 1974; Shimkin, 1974; Bickerton, 1974; NAS, 1975; NAS, 1977; WHO, 1978a-b; FSC, 1978).

Information on the normal life span of test animals is needed to determine certain correction factors which must be taken into account when expressing the final results. Determination of the concurrent spontaneous tumor rate is important because it determines the statistical significance of the disease or tumor rate in exposed groups. As shown in Table 1, the higher the spontaneous tumor rate in controls, the higher incidence of tumors exposed groups must have for them to be statistically significant. Therefore, studies designed to detect weakly active chemical toxicants must be carefully controlled.

If the required matched control group is a vehicle control group or if the toxic effects of the vehicle are unknown, the tester may want to also use a negative or untreated control group to determine whether any effects observed in the vehicle-exposed control group or chemically exposed groups are due to ancillary materials used in the study. Use of these two types of controls should help safeguard against attributing a chronic effect to the test substance when in fact it may be due to the vehicle instead.

b. Positive Controls: A third type of control is the positive control. For purposes of these test standards, this type of control is not required for every chemical but may be required by EPA when it is necessary to ascertain whether the test animals are sensitive to or respond in a predictable manner to known chemical toxicants, to assess the relative potency of certain chemical toxicants and to test the reliability of a laboratory conducting chronic health effects studies (NAS, 1961; WHO, 1969; J.H. Weisburger, 1974;

Sontag, Page and Saffiotti, 1976; NAS, 1977; Sontag, 1977; Page, 1977b; WHO, 1978b). Establishing that test animals are capable of responding in a predictable manner to a known chemical toxicants provides a degree of confidence that tests with unknown agents can be accepted as valid.

As stated above, inclusion of positive control groups will not be required for each unknown chemical required to be tested. However, it may be required or recommended that they be used in relationship to testing of a series of compounds (Sontag, Page, and Saffiotti, 1976; Page, 1977b; WHO, 1978b). Selection of a positive control substance ideally should be on the basis of chemical similarity to the agents under test (Arcos, Argus, and Wolf, 1968; Peck, 1974; NAS, 1975; Sontag, Page, and Saffiotti, 1976). However, in a large testing program, where chemicals having diverse structures are being tested, proper considerations of structure may not be possible. For large-scale testing programs of orally administered agents, the most common oncogens used appear to be N-2-fluorenylacetamide (2-AAF), diethylnitrosamine (DEN), safrole, and 3-aminotriazole. Target organs for 3-aminotriazole are primarily the thyroid and liver, whereas the other three are primarily liver oncogens. DEN and 2-AAF are potent carcinogens, whereas safrole and 3-aminotriazole are less potent. Other compounds used include uracil mustard, nitrogen mustard, urethane, 7, 12-dimethylbenz(a)anthracene, N-dimethylstilbenzmine, and N-methyl-4-diethylaminoazobenzene, N-methyl-N'-nitro-n-nitrosoguanidine and N-(4-(5-nitro-2-furyl)-2-thiazolyl)- formamide.

It should be recognized that there are species/strain differences in response to some positive control substances. Thus, sensitivity of animals in use should be considered in selecting positive control substances.

While the NCI guidelines recommend only a single dose level for positive controls (Sontag, Page, and Saffiotti, 1976), EPA may require that several dose levels be used in order to equate response(s) to procedural design aspects, such as concentration, method of administration and length of exposure. While a low dose of a positive control substance might mimic the weak response of the chemical under study, a higher dose could serve to quickly verify consistency and sensitivity of response of test animals. Use of multiple dose levels of a positive control substance might also allow for semi-quantitative interpretation of results with the test substance.

For some strong carcinogens such as 2-AAF and DEN (Sontag, Page, and Saffiotti, 1976), number of animals required, especially at high dose levels, may only need to be 20-25 per group.

Regardless of the positive control substance used, precautions should be taken to minimize exposure of personnel or other animals in the laboratory. Use and handling of positive control chemicals should be carefully monitored and conform to proper safety precautions.

c. Historical Controls: The fourth type of control group is the historical or colony controls which should include all control animals for a specific strain or breed studied within the most recent five-year period (ILAR, 1976). These controls are a valuable source of information on the normal life span and spontaneous disease and tumor incidence of each individual strain. They cannot, however, replace matched controls because of the accuracy needed in determining the spontaneous disease and tumor incidence (NAS, 1977). Data on historical controls is required to be submitted as a part of the study plan.

7. Route(s) of Exposure

Objective: The standards for selection of route(s) of exposure are set forth to provide the most reliable test system.

In agreement with other standards (Shubik and Sice, 1956; FDA, 1959; Berenblum, 1969; Peck, 1974; NAS, 1975; Canada, MHW, 1975; NAS, 1977; WHO, 1978a; FSC, 1978), EPA is generally requiring that test substances be administered by the route that duplicates or most closely simulates the major known or expected route by which human exposure occurs. This is the accepted method because results are generally directly amenable to evaluation in terms of potential human health hazards.

However, if humans are exposed via several routes, the major route of exposure may not be the most important parameter. In this case, EPA may consider the most important parameter to be the route which is anticipated to be the most sensitive in terms of chronic toxicity. For example, even though greatest exposure to a chemical may be to the skin, the more hazardous exposure may be through inhalation or ingestion. This is due to the fact that the mode of administration influences toxicity at a given site since it dictates concentration at the site (WHO, 1969; J. H. Weisburger, 1975). In deciding on route of exposure, EPA will consider not only human use or exposure but also specific properties of the chemical including its absorption, distribution and metabolism, organs affected, dose, and length of treatment. As will be discussed later, potential safety hazard to laboratory personnel must also be considered.

Each route of exposure has advantages and disadvantages. Weisburger and Weisburger (1967), Arcos, Argus and Wolf (1968), Magee (1970), Canada, MHW, (1975) and Page (1977b) describe in detail various considerations involved in using each specific route.

8. Period of Exposure and Observation

Objective: The standards for selection of exposure and observation periods are designed to make exposure conditions optimal for revealing any long-term toxic effects.

a. Period of Exposure:

i. Oncogenicity Test Standards. The tester must administer the test substance to rats and mice for a minimum of 24 months but no longer than 30 months.

A few powerful chemical oncogens can induce statistically significant incidences of tumor in a short time period and after only one or a few doses (Clayson, 1962; J.H. Weisburger, 1976). However, most chemical oncogens need to be administered continuously for long periods of time before statistically valid conclusions can be obtained (Zwickey and Davis, 1959; NAS, 1961; Magee, 1970; NAS, 1975; Canada, MHW, 1975; Page, 1977b; WHO, 1978b; FSC, 1978). As discussed previously, to maximize sensitivity of the test system and, thus, to enhance confidence in a "negative" result, a test substance must be administered continuously for the greater part of an animal's life span (Tomatis, 1974; Page, 1977b). Present controversy is over testing for a long but finite period or for the full life span of the test animals.

EPA, in agreement with most federal agencies and scientific groups, is requiring use of a long (24-30 months) but finite period of exposure (Berenblum, 1969; Magee, 1970; J. H. Weisburger, 1973; Peck, 1974; Sontag, Page, and Saffiotti, 1976; WHO, 1978b; FSC, 1978). Use of a finite period of exposure has several advantages. Although induced-tumor incidences increase with time, incidences of spontaneous tumors in controls also increase especially during the latter part of an animal's life span. Over the life span of the animal, spontaneous tumor incidences may increase so much as to conceal a true positive (Berenblum, 1969). Sacrificing at a given time enables better evaluation of data since with this method time-at-risk and age are constant for exposed and control groups. Quality of tissue samples are anticipated to be higher in animals killed at a finite time. Tissue quality generally decreases as age increases (Berenblum, 1969). Termination of a study after a given period is more cost effective because it helps facilitate both the pathology examination and evaluation of test data. There will be no need to continue a study simpl because one or two animals have survived for an unusually long time (Berenblum, 1969). There will also be no need for an above average quality and quantity of staff to take care of aged animals.

The Agency is requiring a minimum duration of 24 months for both the rat and mouse. While this duration for rats may not be controversial, its use for mice may be controversial. However, EPA believes that under the hygenic and dietary conditions prescribed by the proposed test standards most strains of mice will have a high survival rate at 24 months. The NCI has found that approximately 75 percent of its control B6C3F1 mice survive to 25-1/2 months of age (Page, 1977b). Such an increase in exposure time will help to maximize the sensitivity of the study. The one assumption being made is that 24 months is adequate time to detect virtually all chemicals which might be potential oncogens. Experimental evidence seems to indicate that very few tumors will be observed for the first time in a study only after 24 months (J. H. Weisburger, 1976).

ii. Non-oncogenic Chronic Toxicity Test Standards.

A. Rodent. Duration of dosing in non-oncogenic chronic toxicity tests for rodents, like for the oncogenicity tests, has long been the subject of controversy. Life span studies in the rodent are recommended by the NAS (1975) for evaluating chemicals in the environment. NAS (1977) cites life span studies in rodent with sacrifice when mortality reaches 80 percent. Schroeder (1973) described the effects of 30 trace elements on life span and found the median life span to be a sensitive indication of effect. Gray (1977) showed that mortality due to chronic progressive nephrosis had a dose-response relationship in a long-term toxicity test.

Toxicological assessment of chemical effect by life time exposure with determination of effects on growth, mortality, patterns of disease, reserve function capacity of organ systems, sensitivity to infection, and median life span provide necessary information for hazard evaluation to man and his environment. EPA is requiring studies of at least 30 months for two reasons:

(1) to determine effects on median life span; and (2) to determine changes in sensitivity with age. EPA believes that use of this long but finite period will allow sufficient information to be collected on both of these effects while allowing certain advantages. These advantages, as discussed in the previous section, include better evaluation of data, better quality tissue samples, and facilitation of the pathology examination and data evaluation.

B. Non-rodent. Duration of non-rodent studies have likewise been a controversial subject. Fitzhugh (1959) cites in the Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics, "Although previous outlines have recommended only one year in dogs, newer information confirms our opinion that insidious changes require 2 years or more to develop in this species." NAS (1977) recommends a 1 to 2 year study in dogs. Loomis (1974) recommended a duration of 2-7 years in non-rodents.

A two year study in the dog represents approximately 10 percent of this species life span. Therefore, in terms of life span, two year dog studies are equivalent to subchronic toxicity testing in rodents. Utility of the 10 percent life span study for subchronic toxicity effects has been aptly demonstrated in the rodent (Hayes, 1975; Boyd, 1972). Historical utility of the two-year study in dogs is aptly demonstrated by their use in setting acceptable daily intake (ADI) for thirty-three pesticides (Vettorazzi, 1975).

Several investigators have found additional effects when dogs were exposed for longer than six months. Braun, Sung, et al. (1977) found changes in some clinical chemistry parameters at 2 years but not at 18 months while investigating long-term toxicity of tetrachlorobenzene. Weil, Woodside, et al. (1971) observed hematologic changes at two years which were not seen at 1 year in dogs exposed to propylene glycol. Histopathologic changes were observed in kidney and liver of dogs exposed to methomyl for 2 years but not at one year (Kaplan and Sherman, 1977). Case, Smith and Nelson (1976) noted central nervous system stimulation in dogs at one year but not at six months after feeding netopam. Herrman, Wiegleb and Leuschner (1977) found side effects in the last weeks of a 12-month experiment which were attributed to exhaustion of fluid and electrolyte reserves in the dogs following exposure to etozolin.

After reviewing this data, EPA believes that a two year study in the dog is necessary to adequately assess the chronic toxicity potential of chemicals for TSCA

Section 4(a) purposes.

b. Period of Observation: Another issue related to the duration of study is whether there should be an observation period between termination of exposure and time of kill. The major reason for recommending such an observation period is to allow time for any reversible lesions to regress and for any irreversible lesions to progress (Sontag, Page, and Saffiotti, 1976; Page, 1977b). This is said to enable the pathologist to better assess any chemically induced irreversible toxic manifestation such as precancerous lesions. Most advocates of this issue recommend a 3-6 month observation period but this is empirical (FSC, 1978). Such a period should be determined by the pharmacokinetic properties of the test substance and the lesions induced. A major difficulty with such an additional period of time is the need to decrease time of exposure to the chemical, thereby, decreasing sensitivity of the test or to increase the duration of the study. EPA is not requiring an observation period but if a testing laboratory wants to include such an observation period, it should include additional groups of animals at the start of a study which can be taken off of treatment early and then observed.

10. Interim Kill

Objective. The standards for interim kill are set forth to allow testers to obtain at their discretion additional information which may help in final interpretation of test results.

EPA is not requiring interim kill of test animals because the proposed test standards are primarily designed to detect differences in type and incidence of toxic effects and not differences in latent periods or pathogenesis of the conditions, because of the increased costs due to the need for more animals and more pathology, and because of the uncertain use of the resultant data in risk assessments. Admittedly, however,information from interim kills may be useful for evaluating the pathogenesis and latent period of induced lesions and for assessing reversible changes (NAS, 1977; FSC, 1978). If the tester wants to include an interim kill in a study, the Agency would welcome such data; however, the number of animals at the start of the study must be increased by the number scheduled to be killed. This is to assure that sufficient numbers of animals are at risk for the long-term exposure to determine if there is a statistically significant difference in number of exposed animals with toxic manifestations compared to control animals.

C. Study Conduct Issues

1. Introduction

As shown in part III of this document, studies designed to detect oncogenicity and chronic toxicity involve extensive investment in animals, materials, personnel and time. Because of this, each test animal becomes a very valuable asset, especially the further the study progresses. Loss of any test animal due to disease or loss of any tissues due to autolysis or cannibalism must, therefore, be avoided. At least two aspects of the conduct of any well-designed chronic toxicity study readily determine if the study will be executed successfully. These are proper and timely observation of each test animal during the test period and proper conduct of the pathology examination.

2. Clinical Procedures

Objective. The standards for clinical observation and tests are designed to detect and assess toxic effects at the clinical level, to help eliminate certain extraneous factors that might adversely influence conduct of the test and/or interpretation of results, and to help ensure the quality of animals and their tissues, thereby, providing reliable animal test systems.

a. Clinical Observations. Appropriately trained employees must observe all test animals at least every 12 hours throughout the test period. Each animal must also be weighed and clinically examined at least once each week during the first 13 weeks of the study and every 2 weeks thereafter.

General clinical observation and examination of test animals is a neglected area in all toxicological assessments (Canada, MHW, 1975; FSC, 1978). Fox (1977) and Arnold, Charbonneau, et al. (1977) provide detailed discussions on clinical monitoring and assessment of animals. In chronic toxicity studies, proper clinical appraisal of each test animal must be directed towards keeping the animal alive as long as reasonably possible while still ensuring that its tissue specimens will provide relevant and useful data (Weisburger and Weisburger, 1967; Canada, MHW, 1975; FSC, 1978). The Agency believes that proper observation of each test animals at least every 12 hours throughout the test period will alert the investigator to early onset of an infectious disease or degeneration of health due to the test substance, will decrease loss of tissue samples due to cannibalism or autolysis, and will help assure that not more than five percent of the animals in each group are lost during the study. Twice daily observation also allows for observing animals during light and dark periods and therefore for detecting photo-effects.

EPA may not accept studies with losses greater than five percent per group because it believes such losses can generally be prevented by proper conduct of the study and because losses greater than five percent decrease the sensitivity of the study. Such a decrease in sensitivity may interfere with the detection of the effect and the interpretation of data especially for weak oncogens. In addition, the losses due to cannibalism or autolysis are often those animals experiencing the effect, e.g., oncogenicity, and thus, the most valuable data may be lost. Arnold, Charbonneau _et al_. (1977), reported that proper observation procedures in their laboratory reduced tissue losses due to autolysis to one percent. EPA believes that the cost of careful and frequent clinical observation and examination is a warranted expenditure in order to provide maximum data for evaluating the test and results obtained.

In addition to daily observations noted above, each animal must be palpated and carefully examined at least once a week for the initial 13 weeks while the animal is growing rapidly and then once every two weeks thereafter (Canada, MHW, 1975; Fox, 1977). Palpation of the animal allows not only for detection of certain tumors but also enables detection of any enlargements of internal organs. These detailed physical examination provide indications as to the general health of the animal and its tumor burden.

Body weights and feed consumption need to be monitored because they are indicators of general health and because this information is needed to evaluate the test (Arcos, Argus and Wolf, 1968; NAS, 1977; Page, 1977b; WHO, 1978b). Changes in body weight of the HDL group in oncogenicity studies are used to determine if this dose is adequately toxic. If the test substance is being administered in the diet, feed consumption data are needed to determine how much is being consumed and therefore, determine the dosage of test substance administered. Body weight may be directly related to decreased food consumption, especially for test substances that are somewhat unpalatable to the exposed animals.

b. Clinical Chemistry. Certain quantitative clinical chemistry determinations including hematology (the only determination to be made in oncogenicity studies), blood chemistry, urinalysis, function tests and residue analysis are to be made on a minimum of eight predesignated rodents in each test group and on all non-rodents. For oncogenicity studies, the tester must perform the hematology determinations at one year and at study termination. For non-oncogenic and combined chronic toxicity studies, the tester must perform the specified studies at least at 3,6,12,18,and 24 months and/or at study termination.

i. General.

The objectives of clinical biochemical tests in toxicity studies are to monitor for disease states in exposed as well as control animals (Street, 1970; Cornish, 1971). These studies provide early indicators of toxicity which lead to identification of target organ(s), as well as evidence of reversibility of toxic effects. The purpose of clinical biochemical testing in chronic toxicity studies is different from the usual diagnostic use in clinical medicine in that in the test situation there is a rather homogenous population of test animals, randomly partitioned into several groups, one of which is a matched control. Variability which is routinely seen in clinical situations can be reduced by controlling test sampling time and conditions (diet, temperature, humidity, lighting).

This study design, coupled with methods which have increased sensitivity and precision, permit statistical comparison between control and exposed groups. These statistical analyses can identify very small group differences which may be within the normal range but are biologically significant (Street, 1970).

ii. Blood Chemistry.

Of the forty plus enzymes and other blood components which have been identified, only a few are functional components of the blood (e.g., thrombin, plasmin, cholinesterase, etc.) (Todd and Sanford, 1976). The majority of enzymes are from tissues and organs which the blood perfuses. The amount of enzyme in the blood at any time is a function of its rate of entry into and loss from the blood and is indicative of the normal or disease state of the organ or tissue being perfused (Todd and Sanford, 1976). The mechanisms proposed for entry into the blood includes organ release due to tissue necrosis, increased membrane permeability and increased production. Removal from the blood is controlled by chemical degradation and excretion. Therefore, elevation of enzymes can be due to increased release from organs and tissues or a decrease in removal from blood.

In a similar manner, other components of blood, i.e., electrolytes, urea, glucose, are regulated by rates of entry into and loss from the blood (Cantarow and Trumper, 1975). Homer Smith is cited (Cantarow and Trumper, 1975) as stating that "the composition of the blood plasma is determined by what the kidneys keep rather than by what the mouth ingests." Thus, EPA believes that it is necessary to examine the readily accessible blood, not only because of explicit information but also because of its translation to the clinical situation.

Necessity for using an operational set or panel of laboratory tests for organ and system evaluation is due to the following functions (Hyde, Mellor and Raphael, 1976). Rate of organ damage (indicated by enzyme release) is related to dose of toxicant and susceptibility of the organ or system to the toxicant. Residence time of the enzyme in blood is determined by rate of loss and can be measured as enzyme half-life. Differences in rates may well account in part for difficulties encountered in clinical chemistry determinations and most certainly suggest that rigid quality control utilizing sensitive and precise methods are necessary for evaluation of these parameters and that multiple clinical biochemical parameters are necessary to fully evaluate organ and system status.

Representative panels are shown in Table 2. Development of automated analysis has reduced the cost for most if not all the determinations. Sample size requirements have been reduced and generally require less than 1 ml of serum, thereby eliminating the need to sacrifice the test animal.

TABLE 2

Representative Clinical Chemistry Panels

Renal Panel
- Blood Urea nitrogen
- Creatinine
- Serum protein and electrophoresis
- Osmolarity
- Calcium
- Magnesium
- Phosphorus
- Uric acid
- Electrolytes

Liver Panel
- Bilirubin, total and conjugated
- Urine for bile pigments
- Aspartate aminotransferase (GOT)
- Alkaline phosphatase
- Serum protein and electrophoresis
- Gamma-glutamyl transpeptidase
- Leucine aminopeptidase
- Alanine aminotransferase
- Ornithine carbamyl transferase

Muscle Heart Panel
- Creatine kinase
- Aspartate aminotransferase (GOT)
- Lactate dehydrogenase

Hypertension Panel
- Renal panel plus:
- Serum electrolytes
- Acid-base balance: blood pH, P_{co_2} bicarbonate
- Lactate dehydrogenase
- Triglycerides
- Cholesterol

Pancreatic Panel
- Serum amylase
- Urine amylase
- Serum lipase

EPA is of the opinion that clinical biochemical parameters performed with adequate sensitivity and precision and evaluated by appropriate statistical tests are necessary for complete assessment of chronic toxicity.

iii. Urinalysis.

EPA is also requiring routine urinalysis. Such routine urinalysis may be the most used and abused procedure in toxicological evaluation. One frequently finds statements as to the doubtful usefulness of routine urinalysis (NAS, 1977). It should be emphasized that routine semiquantitative urinalysis is a screening procedure and only that. As with any screening test, a follow-up with a quantitative determination is required when a positive is found.

As pointed out earlier, the analysis of blood components is an useful indicator of renal function. Urinalysis is required to detect early kidney damage since enzymes of renal origin do not appear to any extent in blood (Todd and Sanford, 1976). Urinary protein correlates well with renal pathology (Gary, 1977). Urinary glucose is an indicator of pancreas function and of proximal tubular function of the kidneys (Cantarow and Trumper, 1975). Urinary excretion is also a major pathway for elimination of xenobiotics (Cantarow and Trumper, 1975).

After reviewing the available data, EPA agrees with Berndt (1976) that routine urinalysis is not only very beneficial but necessary for complete toxicological evaluation.

iv. Function Tests.

Function tests like blood chemistry tests are necessary to detect and characterize toxic effects as expressed by abnormal biochemical and physiological functions (Benitz, 1970). These tests allow detection of all types of changes including non-morphological changes (NAS, 1975). EPA realizes that it is impossible and not practical to give an exhaustive list of all function tests or to make specific recommendations for certain classes of chemicals. It also agrees with Benitz (1970) that it is more judicious to select the most meaningful tests depending upon the toxicity results in subchronic toxicity studies or in the early phases of the chronic toxicity study. EPA believes, however, that certain organ systems (i.e., liver, kidney, pulmonary and cardiovascular systems) must be routinely monitored via some function tests because of their key positions in the metabolism, distribution and/or excretion of toxic substances. Such key positions increase the chance that these systems will be affected by the test substance.

v. Residue Analysis.

The non-oncogenic and combined chronic toxicity studies require measurement of levels of test substance and certain metabolites in plasma, urine, feces and target organs. With such data, comparisons can be made between the amount of concentration of a test substance or its metabolite(s) in a target tissue or body fluid and the corresponding level of toxicity, it can be used to determine whether a steady-state concentration is achieved, and it will give some general understanding of the absorption, disposition and elimination of the test substance and how these parameters vary with concentration and between species. These types of information in turn allow more complete and accurate risk assessments to be made. They also form a bridge between animal studies and human exposure (Burchfield, Storrs and Green, 1977). EPA believes that the importance of the data supplied by residue analysis studies outweigh its cost.

3. Pathology Procedures.

Objective. The standards for pathology procedures are set forth to ensure that an in-depth pathology examination is done to detect minute as well as more obvious toxic changes.

a. General. The tester must conduct detailed necropsy and histopathology examination of all animals with approximately thirty (30) to forty (40) tissues routinely examined microscopically.

It is essential in chronic toxicity studies to include adequate consideration of pathology (Shubik and Sice, 1956; Magee, 1970; Roe and Tucker, 1974; FSC, 1978). Final success of an entire study depends on the current diagnosis and interpretation of the significance of any lesion(s) noted both of which are part of the pathology evaluation. To plan and conduct an animal bioassay meticulously and then to permit an inadequate pathology evaluation would be ludicrous. On the other hand, no amount of subsequent pathology interpretation will extract useful information from poorly planned or executed studies. Two essential criteria for success in the pathology examination itself are properly prepared tissue specimens and qualified personnel (Barnes and Denz, 1954; FDA, 1959; Weisburger and Weisburger, 1967; Page, 1977b; FIFRA, 1978; WHO, 1978b; FSC, 1978). A lack of either will predispose a study to failure.

A complete pathology examination consists of several steps: macroscopic examination (necropsy), histologic preparation of tissues, microscopic diagnosis, recording and tabulation of lesions, and evaluation of the test (Page, 1977b). These procedures represent a major aspect of a chronic toxicity study both in terms of time and expense. In the NCI bioassay program, approximately 40 percent of the overall cost of a study is for the pathology examination. One-fourth to one-third person-years of a pathologist's time is needed just to examine the 15,000 to 20,000 tissues from one study (Page, 1977b). The requirements are even greater for the proposed TSCA test standards. Because of economic and time considerations and because of the shortage of qualified staff (GAO, 1978), controversy has developed over how to cut back on the pathology examination without drastically reducing the degree of confidence in the final interpretation of the data (Sontag, 1977). As discussed below, EPA does not believe that for TSCA Section 4 needs the amount of pathology can be reduced without compromising test results.

b. Gross Necropsy. There seems to be complete accord that a detailed necropsy must be performed on each test animal and that all of its major organs and tissues must be preserved (FDA, 1959; Weisburger and Weisburger, 1967; WHO, 1969; FDA, 1971; Peck, 1974; Sontag, Page, and Saffiotti, 1976, NAS, 1977; WHO, 1978b; FSC, 1978). Extent and accuracy of the pathology findings and, thus, those of the study are dependent on extent and accuracy of the gross necropsy. It represents the last and most important chance to obtain biological evidence.

To obtain maximum usable information from a study, animals should be necropsied and tissue samples taken immediately after death or kill. Because this is not practical in all cases, the Agency has reached a compromise that the necropsies be done within 16 hours of death. EPA believes that if the time of necropsy is increased further certain important tissues will be lost to autolysis and also that the fine details of other tissues will be lost. Thus, the sensitivity and the reliability of the study data will be decreased.

c. Microscopic Examination. While there seems to be little controversy regarding the need for a careful and thorough gross necropsy, considerable disagreement exists as to number of animals and number of tissues from each animal that should be examined microscopically. Many guidelines use such terms as "complete" or "adequate" examination, leaving selection of animals and tissues up to the responsible pathologist's judgment (FDA, 1971; Magee, 1970; WHO, 1961). Most recently, a series of committees have recommended that as a minimum routine procedure, microscopic examination be done on all major tissues and gross lesions for only the high-dose and control groups supplemented by gross lesions and target organs from other dose groups (Peck, 1974; NAS, 1977; FIFRA, 1978, FSC, 1978). This type of recommendation places primary reliance upon the gross necropsy examination for detection of lesions and is based on the assumption that induction of lesions is dose dependent. Peck (1974) recommends complete microscopic examination on a representative number of animals from the high-dose and control animals supplemented by target organs from other dose groups. NCI has stringent requirements in that all test animals must undergo extensive microscopic examination (Sontag, Page, and Saffiotti, 1976).

Controversy over number of tissues to be examined is also considerable. Abrams, Zbinden and Bagdon (1965) felt that 18 different tissues should be routinely examined while the WHO (1961) recommended an initial examination of only five organs (lungs, liver, spleen, kidneys and urinary bladder), plus organs showing gross lesions. The NCI bioassay program was the first to introduce an expanded protocol that required microscopic examination of approximately 30 tissues sections plus blood smear, tissue masses, and gross lesions from each test animal (Page, 1977b). Zbinden (1976) believing that a compromise was needed between an all-encompassing evaluation of all possible tissues and a superficial examination, assigned tissues into priority classes according to the frequency with which morphological changes are likely to occur. Fears and Douglas (1978a-b) have also proposed for comment suggested procedures for reducing the pathology workload.

EPA is in agreement with NCI that all test animals must be thoroughly examined microscopically. In the Agency's opinion, all test animals must be examined thoroughly since there can be changes in organ specificity as the dose level is varied. Throughout the development of the chronic toxicity standards, it has been the policy of EPA to try and maximize the sensitivity of the test system, and, thereby, enhance confidence in its ability to detect chemical toxicants. One of the most crucial ways of accomplishing this is by thorough microscopic examination of each test animal. This enables detection of not only gross lesions but also detection of early small lesions, those in small organs and those that have metastasized and are in a minute form.

D. Data Collection and Reporting Issues

1. Final Report

Under the proposed test standards, the Agency is requiring submission of an extensive final report. The sponsor must submit a full and detailed report, generally in tabular or graph form, of all test conditions, all observations made on the animals, and the complete data analysis. Summaries only of the results are not acceptable. Examples of the data that must be collected are information pertaining to changes in body weight, food consumption, dose administered, diseases and treatments, time of death, method of kill or cause of death, presence of any pathological lesions and time of first observation, if known, all signs of toxicity and their time of onset, regression or progression of any lesion, irreversible lesion incidence by tissue and type, multiplicity of specific lesion types, and pathology diagnosis.

There are several purposes for such detailed data requirements. Foremost is EPA's need to be able to check the accuracy of the sponsor's analysis and to make its own independent evaluation of the study. Without such information, EPA scientists simply could not accomplish these tasks. It is commonly recognized that inadequate documentation is one of the most difficult problems confronting scientists in evaluating chronic toxicity results (Berenblum, 1969; Sontag, Page, and Saffiotti, 1976; Page, 1976; Page, 1977a-b; FSC, 1978). Neither summaries of studies nor the current journal style of publication provide the full report of test conditions and animal observations that is necessary if another scientist is to evaluate the empirical and analytical bases for the conclusions reached by the sponsor. The ability to engage in such reviews is particularly important in light of recent discoveries that call into question the validity of many toxicity studies submitted to EPA and the Food and Drug Administration in the past. (For further discussion of this point refer to the preamble to the proposed "Good Laboratory Practice Standards for Health Effects.")

Detailed reports serve other functions as well. They will facilitate the peer review process, thereby helping to assure that reliance will be placed upon the studies which merit it. Moreover, as additional scientists review the data, the fresh perspectives they bring may lead to new insights into the data and a better understanding of the study's significance. And, the knowledge that the data will be subject to scrutiny will provide an incentive for the sponsor to produce the best data. Finally, EPA will be assured that the necessary information will be available for all chemicals that are tested, thus facilitating efficient and reliable comparison and evaluation of different studies.

Review of the data will focus on three elements: study design, study conduct, and the conclusions drawn from the data. Examination of the first two elements will provide the first major indication of the study's worth; if a study is not properly designed and conducted, the resulting data will be inadequate, unreliable, or insufficient to interpret. Without proceeding any further, the reviewer will already know he must be suspicious of the data base and the validity of the study. Roe and Tucker (1974) have listed some of the common faults found in chronic toxicity tests that can be found by analysis of study design and conduct: (1) inadequate randomization; (2) unintentional variations such as differences in room temperature and humidity; (3) high loss of animals without post-mortem examination; (4) poor records of necropsy findings; (5) use of non-standard post-mortem techniques; and (6) failure to match microscopic findings with macroscopic ones.

A reviewer would then look at the various data in order to assess the validity of conclusions reached by the sponsor, i.e., whether the test clearly indicates a toxic response(s), whether the results are suspicious or inconclusive; or whether there is no evidence of toxicity (Page, 1977b). To do this, the totality of the biochemical, clinical pathology, and statistical evidence gathered on the test substance during the study must be available (D'Aguanno, 1974; FSC, 1978).

In summary, extensive documentation is necessary to assess the validity and reliability of each study and to allow independent scientific analysis of the results. For the majority of chemicals to be tested under the proposed chronic toxicity standards, the decision as to their chronic toxicity potential will probably be based solely on evidence obtained from one such study, making it especially imperative that each study meet certain scientific criteria. Hence, the need to incorporate data collection considerations into the original study design and to require detailed reports.

2. Interim Report.

Besides requiring submission of a detailed final report, EPA is also requiring submission of quarterly interim reports. The purpose of these reports is three-fold: (1) to monitor the progress of a study; (2) to learn of significant findings of such parameters as survival, weight changes, clinical test results and accumulative incidence of tumors and toxicity; and (3) to learn of any catastrophic events which might affect the quantity or quality of data and thereby, affect the interpretation of the data or completely invalidate a study. Such monitoring of studies will allow for communication between EPA, the sponsor and the tester during the study. More importantly, such monitoring will make EPA aware of any possible imminently hazardous chemicals or potentially hazardous chemicals which should be considered for regulation before the final report is submitted. EPA believes that the best way to accomplish this is by requiring submission of summary reports on a quarterly basis.

E. Good Laboratory Practice Issues

1. Introduction

Along with the test standards, the Agency has proposed Good Laboratory Practice (GLP) standards to assure the quality and integrity of data submitted from health effects testing, including chronic toxicity studies (Chapter 40, Part 772, Subpart B, section 772.110-1 of the Code of Federal Regulations). The GLP standards set forth criteria for such matters as test substance characterization, general personnel requirements, administration of testing programs, facilities and equipment, facilities operation, and general study design and conduct. These are only general criteria, however, that apply to all health effects laboratory testing under TSCA. Specific, more detailed, requirements for good laboratory practice are to be developed in test standards for particular effects testing, as appropriate. In the chronic toxicity test standards several of these more detailed criteria have been proposed.

2. Personnel

The Agency considers adequate training and experience a key factor in assuring the quality of data generated. Therefore, the Agency has adopted stringent requirements for specific key personnel involved in the design, conduct and analysis of chronic toxicity studies. The Good Laboratory Practices Standards for Health Effects (Section 772.110-1) proposes general educational and experience requirements for the study director, and other personnel while the chronic toxicity test standards (Section 772.113-1) set forth specific qualification requirements for the pathologists, veterinarians and certain technical employees.

As stated previously, two essential ingredients to success in the pathology phase of a study are quality tissue specimens and qualified personnel. Qualified personnel includes everyone involved in the study. Key to a successful animal care operation is a well-supervised, well-trained and motivated animal care-taker staff interested and concerned about the health of individual animals and their role in quality research (Page, 1977a-b; NAS, 1977). Obtaining quality tissue specimens is dependent in part on the humane care given to animals and astute observations of the veterinarians and animal care-taker staff. Well qualified histology technicians are essential to obtaining professionally prepared tissue slides. Careful microscopic evaluation cannot make up for lost animals or for inadequate gross necropsy and histologic preparation. In simple terms, the pathologist reviewing tissues slides can report only lesions placed before him. Need for highly qualified pathologists is equally important and cannot be over emphasized. Final analysis of a study is based primarily on pathology findings, and the extent and accuracy of these findings depend on the qualifications of the pathologist.

3. Animal Care and Facility

Quality of test animals, animal facilities and animal husbandry practices contribute in no small manner to successful outcome of a study (Who, 1978, FSC, 1978). Again, long-term animal studies are too important and too costly to begin a study with animals of inferior quality (Sontag, Page and Saffiotti, 1976). An appropriate animal facility with adequate environmental controls is an essential element in assuring that animals do not succumb from causes extraneous to the study. The facility must be designed and maintained to meet the high standards of animal care and chemical and biological hazard controls required for reliable and safe chronic toxicity studies. Operation of the facility must strive to prevent entrance of extraneous factors at all levels of containment, from facility to animal room to individual cage (Page, 1977a). Several publications are available that provide detailed information and recommendations on animal facilities and their operations (Arcos, Argus and Wolf, 1968; Canada, MHW, 1975, NAS, 1976; Sontag, Page, and Saffiotti, 1976; NAS, 1977).

Successful outcome of a study is also dependent upon the quality and extent of animal care and husbandry (Page, 1977b). Primary goals of animal husbandry practices are to promote health and humane care of animals and to control environmental variables among individual animals and test groups. Page (1977b) has listed some of the most important husbandry factors that can influence oncogenicity tests (Table 3). EPA believes that these factors can be controlled or prevented by routine practice of strict hygiene and disease prevention measures and close clinical observation.

Table 3*

Husbandry Factors That Can Influence Chronic Toxicity Tests

1. Infectious Diseases
    a. Microbiologic
    b. Parasitic
2. Chemical Pollutants in
    a. Feed
    b. Water
    c. Bedding
    d. Air
3. Operations Management
    a. Cannibalism
    b. Autolysis
    c. Vermin Infestation
    d. Diet
    e. Prevention of Cross-Contamination

*Page, 1977b

4. Dietary Requirements

Objectives. The standards for selection of diets are set forth to help ensure the quality of test animals throughout a study and to eliminate certain extraneous factors that might influence conduct of a study and interpretation of results.

a. Diet: The tester must feed test animals standardized diets. A standarized diet for rodents is specified in the test standards.The diet is a very significant environmental source of variance in animal research studies including long-term toxicity studies (Barnes and Denz, 1954; FDA, 1959; NAS, 1961; Roe and Tucker, 1974; NAS, 1977). It helps determine the general health and longevity of test animals; it affects sensitivity of the test system; and it affects reproducibility of the study. The specific diet fed to test animals must meet all of their nutritional requirements, be palatable and be free of toxic or infectious microorganisms in order to promote good health and longevity.

A negative relationship between obesity or a low level of nutrition and longevity has been shown in humans (Armstrong, Dublin, et al., 1951) as well as in various laboratory animal species (Berg and Simms, 1960; Lane and Dickie, 1958; Silberberg and Silberberg, 1955; McCay, Crowell, et al., 1935; McCay, Maynard, et al., 1939; Nolen, 1972; Ross, 1961; Ross and Bras, 1975; Ross, 1977). Onset of age-associated changes in kidney function as indicated by decreased protein use and increased PAH transport was delayed in animals fed a restrictive diet (Tucker, Mason and Beauche, 1976). Prevalence and severity of kidney lesions were reduced when caloric intake was decreased (Ross, 1976). Incidence of myocardial and prostatic diseases could be altered by diet composition (Ross, 1976) and a similar relationship was observed between diet and glomerulonephropathy.

Diets are known to affect the sensitivity of the oncogenicity test in two ways. Experimental evidence has shown that both caloric value and individual dietary components and contaminants alter markedly the induction rate and progression of chemically induced and spontaneous tumors in the rodent (Homburger, 1974; Newberne and Rogers, 1976). A series of papers have been published by Ross and his associates showing interactions between dietary nutrient concentrations and incidence of neoplasms (Ross, Bras and Ragbeer, 1970; Ross and Bras, 1973; Ross and Bras, 1975; Ross and Bras, 1976; Ross, 1977;). Dietary effects on induction rate and tumor progression may be due to changes in metabolism, changes in intestinal flora or changes in the host's immunological processes (Newberne, 1974). Test results suggest dietary factors may have as much influence on the incidence of lesions in animals involved in long-term studies as some test substances being evaluated. Accordingly, it is essential to include the diet as an environmental factor to be controlled in long-term studies. This is particularly critical if test results are to be replicated or if data generated at different laboratories are to be compared.

b. Standardization of Diet: The Agency, along with certain scientific groups, is concerned not only with regard to effects that the diet has on test sensitivity but also in being able to standardize testing conditions (Newberne, 1974; NAS, 1975; Canada, MHW, 1975; NAS, 1978; FSC, 1978). Standardization of the diet will enable more reliable comparisons to be made between test substances and between test systems.

Three types of diets are available for use in long-term rodent studies. Closed formula rations are made from natural ingredients and are the normal commercially available rations. Information on ingredient composition of closed formula rations are not readily available since they generally are trade secrets. Since they are made from natural ingredients, there can be considerable variation in nutrient concentration as sources of ingredients are changed. These rations are the most readily available, the most widely used and generally thought to be the most economical.

Open formula rations contain both natural ingredients and ingredients of varying degrees of refinement. They differ from closed formula rations in that both quantitative and qualitative ingredient composition is readily available and can be adjusted to meet specific needs of individual testers. Uniformity of these rations can be assured from lot to lot.

Synthetic diets are formulated only with specified chemicals of known refinement. The major problem with formulating such diets is lack of knowledge as to the complete nutrient requirements of any test animal. They are also expensive and generally not very palatable. Their major advantages are absence of naturally occurring contaminants and complete standarization of ingredients (ILAR, 1976).

Open formula rations most closely fill the need to maximize testing conditions and to standardize the diet and hence are proposed by EPA. Requirement of an open formula ration is not anticipated to significantly increase the cost of long-term animal studies (see Section II). Experience of NIH has shown that their cost for rations has decreased since they began using open formula rations. No shortage of diet is anticipated when Section 4 rules are promulgated since several companies are selling the ration and more are expected to begin selling it.

5. Contaminant Analysis Requirements

Objective. The standards for contaminant analysis are designed to eliminate certain extraneous factors that might influence conduct of a test and interpretation of results.

Contaminants. The tester must analyze feed and vehicles, if any, for certain specified contaminants.

Contaminants can be introduced with either one or more ingredients in a diet or vehicle or through diet and vehicle mixing apparatus (NAS, 1978). These may be industrial contaminants such as the polychlorinated biphenyls and insecticides or naturally occurring contaminants such as aflatoxins. The objective for analyzing for these contaminants is to establish a profile of the kinds and amount of each contaminant, test animals are exposed to during a study. If unacceptable concentrations are detected, a change in ration or source may be indicated. These data will also be utilized when test results are evaluated to ascertain any effect they may have on interpretation of results.

The list of contaminants provided in Appendix A (Section 772.113-1 of the test standards) are known to interfere with long-term chronic toxicity studies (ILAR, 1976). The list originally resulted from a four year surveillance study conducted by the National Center for Toxicological Research. All of these contaminants were detected in analytically significant quantities in the diets used. The presence of such agents in animal feed or vehicle has serious implications. Indeed complex interactions between critical levels of given contaminants might singely or in concert with other test material produce results which are at best unreliable or at worst misinterpreted. EPA believes that the maximum permissible levels given in Appendix A are realistic and based on scientific evidence.

6. Safety and Health Standards

EPA recognizes that long-term toxicity studies especially oncogenicity studies have inherent risks associated with them. These include possible contamination of laboratory facilities, laboratory personnel and the general environment by potential oncogens and toxicants (Sansone, Losikoff and Pendleton, 1977a-b; Page, 1977b; IARC, 1978). Once such events are detected it is too late to undo any exposure that may have occurred. Therefore, proper precautions and codes of practice must be followed throughout a study. The Agency recommends that guidelines developed by the DHEW Toxicology Subcommittee for Carcinogen Standards (August, 1978) and those developed by the International Agency for Research on Cancer (IARC, 1978) be followed.

Other publications dealing with this subject have been prepared and include the following: The National Cancer Institute Safety Standards for Research Involving Chemical Carcinogens (1977); the National Center for Toxicological Research Carcinogen Standards (1978) and the Code of Practice for the Safe Handling of Chemical Carcinogens in Research Establishments (UK, 1978). hese sources may be used for additional guidance.

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III. ECONOMIC ASPECTS OF THE PROPOSED CHRONIC HEALTH EFFECTS TEST STANDARDS

A. SUMMARY

The estimated costs of testing a chemical according to the proposed standards are provided in Table 1 below. 1/

TABLE 1

Summary of Costs

	Oncogenic Tests	Chronic Tests	Combined Tests
Fixed Costs:	$ 45,000	$ 45,000	$ 45,000
Variable Costs:			
Animal Procurement	$ 2,500	$ 10,300	$ 12,000
Rations	1,300	1,500	2,400
Animal Care	96,300	270,800	339,500
Clinical Laboratory Tests	2,600	21,100	24,700
Microscopic Examination	90,000	75,000	152,100
Necropsy and Histological Preparation	150,000	105,000	212,600
Total Variable Cost	$342,700	$483,700	$743,300
Total Cost	$387,700	$528,700	$788,300
Prechronic Testing Costs:	50,900	100,500	125,900
Total Costs including Prechronic	$438,600	$629,200	$914,200

1/ The following terms will be used throughout this chapter:

Term	Refers to
Oncogenic	Oncogenic effects test standard
Chronic	Non-oncogenic chronic toxicity standard
Combined	Combined chronic effects test standard

Most estimates of testing costs provide only a total cost for a test, and give little or no detail on costs for subparts of the test. EPA has adopted a more detailed approach in order to provide a basis for:

1) discussion and improvement of the cost estimates, and
2) calculation of the costs of alternatives to the proposed test standards.

The following points, however, regarding the cost estimates should be emphasized:

- The estimated costs are those of meeting the minimum requirements of the test standards. Actual testing costs will be greater to the extent that optional testing is performed.

- Actual costs of long-term testing will vary substantially, depending on a number of factors, including, for example, the chemical tested, laboratory performing the test, number of dose levels, species, route(s) of exposure, extent of pathology conducted, duration of tests, inclusion of ancillary studies, and other factors.

- As indicated by the detailed calculations in the sections which follow, EPA's cost estimates are calculated based on a number of assumptions concerning labor productivity and wage and overhead rates. These assumptions represent a consensus judgment among EPA oncologists, toxicologists, and pathologists derived from personal experience in conducting chronic health effects testing. In cases where published numbers or other documented numbers were judged reliable by EPA, these numbers were used and are referenced accordingly.

- Costs of Good Laboratory Practice (GLP) items in the test standards do not receive explicit treatment in most cases in the estimates, and thus are included as part of overhead.

- Many cost items are included as part of overhead in the estimates and therefore do not receive explicit treatment. EPA solicits comment as to areas which merit explicit treatment.

- Section 4 test rules will supply additional information (e.g. route(s) of exposure) and may modify the test standards to suit particular chemicals. EPA will develop cost estimates to account for these additions and modifications at the time test rules are proposed and promulgated.

The above costs are on a per chemical tested basis. The total annual cost of TSCA Section 4 test rules requiring these tests will depend on the per chemical costs and the identity and number of chemicals tested for particular effects. The latter items are undecided and thus it is difficult to estimate the testing costs that will result. If one assumes that approximately 15 to 30 chemicals a year will be tested under Section 4 test rules using these particular test standards, and test costs will range from approximately $400,000 (for an oncogenic study where prechronic testing has already been performed) to $900,000 (for a combined study where prechronic testing is required), the total annual cost is estimated to range from $6 to $27 million.

B. METHODOLOGY AND ASSUMPTIONS

1. Methodology

Costs were determined by separating each of the standards into the following subparts and estimating the cost of each:

- Fixed Costs (Section C)
  - Protocol Design and Study Plan Submission
  - Project Management and Preparation of Final Report
  - Statistical Analysis
- Variable Costs (Sections D-2, E-2, F-2)
  - Animal Procurement
  - Rations
  - Animal Care
  - Clinical Laboratory Tests
  - Microscopic Examinations
  - Necropsy and Histological Preparation
- Prechronic Testing Costs (Sections D-3, E-3, F-3)

Calculation of costs in each of these subparts consisted of three elements:

- Determination of the requirements of the rule;
- Determination of assumptions concerning labor productivity, wage rates, etc.; and
- Calculation of cost.

Costs were then summed to give the total for each of the test standards.

2. Assumptions Concerning Study Design

The cost estimates are designed to estimate the cost of satisfying the minimum requirements of the standards. EPA recognizes that some sponsors may choose to perform testing in addition to what is assumed for cost estimation. This testing is regarded in these estimates as optional and is not included. EPA solicits comment as to whether any of this additional testing is really not optional in the sense that sponsors would be induced by EPA regulatory requirements, particularly performance requirements, to perform this testing.

A number of assumptions about key variables were made in estimating the costs of the test standards. The key variables and the assumption made for each test standard are discussed below. Each section first sets forth the requirements of the standard and then explains how the item is treated in the cost estimates. Where EPA expects that sponsors will perform the testing in a given manner, this is also noted.

a. Species

The tester must use the following species for each test:

Oncogenic: at least two rodent species, the laboratory mouse and rat

Chronic: at least two mammalian species, a laboratory rat and a non-rodent

Combined: at least three mammalian species: two rodent species, the laboratory mouse and rat, and a non-rodent.

The dog is recommended as the non-rodent. Alternate species may be utilized if justified to EPA. The cost estimates are based on the minimum required number of species for each test and have assumed use of the mouse, rat, and dog. EPA believes that the large majority of testing will be conducted with these species because of the requirements and recommendations of the standards, and because of the scientific and economic advantages of these species.

b. Group Size

The minimum group size requirements of the test standards are as follows:

oncogenic: 50 rodents per group
chronic: 58 rats per group, 6 dogs per group
combined: 50 mice, 58 rats, and 6 dogs per group.

The standards require that when interim kill is planned, the number of animals at the start be increased by the number of animals scheduled to be killed before completion of the study.

The cost estimates are based on the minimum allowable group size and assume no interim kill. In assuming 58 rats per group, EPA assumed that the minimum of 8 rats per group will survive the clinical laboratory tests and need not be sacrificed (see Section II for further discussion).

c. Control Groups

The standards require the use of matched control groups. 1/ If the test substance is administered to the exposed groups by the use of a vehicle whose toxic properties are not known, the tester may, at his discretion, use a negative (untreated) group. 2/ The EPA may require a positive control group 3/ for particular chemicals when the sensitivity of the test animal cannot be documented for the chemical class to which the test substance belongs.

The cost estimates assume that only a matched control group will be used. The rationale for exclusion of the cost of negative (untreated) and positive control groups is as follows:

- The addition of a negative control group to a study would increase the cost by a considerable amount. For this and scientific reasons, EPA expects that if a vehicle is used, the vehicle chosen will be well-characterized and therefore a negative control group will not be used in most cases.
- A positive control group may be required by EPA. Where this requirement is specified in a particular test rule, the cost of this group will be included in the cost estimates accompanying the particular test rule. Both because of the considerable increase in cost due to positive controls and because of the safety problems associated with using known toxic agents in the laboratory, EPA expects that the use of positive control groups, where not required by EPA, will be relatively infrequent and therefore has not included their cost in these generalized cost estimates.

1/ A matched control group is identical in every respect to an exposed group except that it is not exposed to the test substance. If a vehicle is used in administering the test substance, the matched control group would receive the vehicle but not the test substance.

2/ A negative (untreated) control group is a control group which receives neither the test substance nor a vehicle.

3/ A positive control group is a control group which does not receive the test substance but instead receives a known toxic agent. When used, the positive control group serves as an internal quality control group to ascertain whether the test animals are sensitive to known toxic agents and whether the test strain or species reacts similarly to another strain or species when exposed to the same known standard toxicant.

d. Route(s) of Administration

The routes of administration will be specified in the individual test rules and EPA's cost estimates accompanying those test rules will reflect the route(s) of administration. Since most studies are expected to be feeding studies, the cost estimates presented here have been based on the costs of a feeding study.

e. Duration of Treatment

The test standards require:

oncogenic: 24-30 months for mouse and rat (24 month minimum)
chronic: minimum of 24 months for dog, 30 months for rat,
combined: minimum of 24 months for mouse and dog, 30 months for rat.

The cost estimates are based on the minimum allowable test period. EPA anticipates most testing will conclude after this minimum period.

f. Number of Dose Levels

The standards require:

oncogenic and chronic: at least 3 dose levels (in addition to controls)
combined: at least 3 dose levels (in addition to controls) for mouse and non-rodent; at least 4 or 5 dose levels (in addition to controls) for rat.

The cost estimates use 3 dose levels for all tests and species (plus controls) except for the combined test for the rat because this is the minimum required. In the combined study for the rat, the estimates assume that 4 dose levels (plus controls) will be used as often as 5 dose levels (plus controls). An average of these two levels is therefore used for these cost estimates. This approach is employed because EPA is unable to predict at this time how often 4 dose levels will be required versus how often 5 dose levels will be required.

g. Number of Animals Purchased

Although the standards do not address the issue of the number or percentage of animals in control groups that must survive to termination of the study, good survival is clearly important. One of the ways to accomplish this is through starting the study with healthy animals. EPA believes many laboratories will order more animals than will be used in the study for prescreening purposes. These cost estimates assume test laboratories will purchase 20% more animals than actually started with the test to assure starting the study with healthy animals.

3. Costing Assumptions

A number of specific cost assumptions were made in estimating the costs of each test standard. The cost assumptions which apply to all three test standards are explained below.

a. Overhead Rate

An overhead rate of 100% is applied to all labor costs in the cost estimates. This includes costs that testing laboratories may separate into overhead, general and administrative, and fee or profit. There are three areas in which this assumption may be limited:

1) Overhead is used in the estimates to capture all costs not explicitly estimated. In some cases, it may be desirable to develop a specific cost estimate for items now lumped into overhead.

2) Overhead rates specific to particular parts of the tests could be used, as opposed to a uniform rate. If significant differences in overhead exist for different parts of the test, use of specific rates could improve the cost estimates for particular parts of the standards and for variations in the rule requirements.

   EPA is uncertain as to whether significant overhead differences can be identified for different parts of a test. Some factors suggest a rough uniformity of overhead - e.g., highly-paid pathologists work with expensive microscopes. Other factors suggest that use of a uniform rate may be inappropriate - e.g., social security expenses and the cost of running a payroll system vary more with the number of employees, than with the wage bill, and thus do not increase proportionally with wage rates. Given other causes of variations in the cost estimates, it is doubtful that use of specific rates will significantly improve the estimates.

3) Assuming a single overhead rate is appropriate, then 100% may still not be the best estimate. However, the fact that the cost estimates here are within 15% of those of the National Cancer Institute when adjusted

for differences in the standards (see "Cost of Alternative Standards" section) lends support to the belief that the use of 100% (in conjunction with the manpower estimates and wage rates used here) is probably reasonable.

b. Pathologist Requirements

The standards provide that a Board-Certified or Board-Eligible pathologist with a minimum of three years of experience in pathology of the species of laboratory animals to be used, may directly supervise other doctorate pathologists for conducting certain procedures. The cost estimates do not specify separate categories of pathologists with different salary rates, but merely use one category and rate. The salary rate used is intended as an average of the different rates that exist for these categories. Comment is solicited as to whether EPA should attempt to use a weighted average.

c. Veterinarian

The standards require that a veterinarian who is Board-Certified or eligible for certification by the American College of Laboratory Animal Medicine, and who has a minimum of two years of experience in laboratory animal science, be responsible for the health status and care of all test animals. The cost of the veterinarian is included in the overhead estimate, and not costed explicitly.

d. Technical Employee (Necropsy)

Necropsies must be performed by a pathologist or by a technical employee under the personal supervision of a pathologist. Such technical employee must be certified by the American Society of Clinical Pathology (HTASCP), or have equivalent training and experience. The cost estimates assume that most, if not all, of the necropsies are performed by such technical employees. The time required for supervision by pathologists is included in the "Microscopic Examination" section; the assumption concerning the number of sections per year that a pathologist can examine microscopically is based on 4 hours per day at the microscope with a portion of the remaining time to be spent in necropsy supervision.

e. Technical Employee (Animal Care)

Technical employees responsible for the daily observation and care of test animals must be certified or eligible for certification by the American Association of Laboratory

Animal Science (LTAALAS), or have equivalent training and experience. The cost estimates assume that these technical employees shall conduct the twice daily observations and the biweekly clinical examinations, but that the feeding and cage cleaning will be conducted by personnel with less training (termed "technicians" in the estimates).

f. Salary Rates

The cost estimates assume the following average annual salary rates for each type of employee:

Pathologist	$50,000
Senior Statistician	$40,000
Intermediate Statistician	$24,000
Junior Analyst	$15,000
Technical employee (HTASCP certified) for necropsy	$15,000
Animal weight, clinical exam, and daily observation technical employee (LTAALAS certified, or eligible)	$12,000
Technician to clean cages and feed animals	$ 8,000

g. Variable Costs

All variable costs are assumed to be strictly proportional to the size of the experiment; e.g., doubling the number of animals doubles the variable costs. While this assumption is probably not valid over a large range, for a small range (± 25%) it may be more reasonable. This range is sufficient to capture the variation in cost for many of the possible modifications to the standards (See "Cost of Alternate Standards" Section H, Table 5).

h. Caging

These estimates assume 5 mice per cage and 2 rats per cage. Two rats per cage is an average, with actual caging practices varying from 1 to 3 rats per cage. Since many of the animal care operations are costed on a per cage basis, the effect of this assumption is to make animal care for rats much more costly than for mice and possibly more costly than will be the case in practice.

i. Animal Space Requirements

Animal space requirements are included as part of overhead. This was done for two reasons:

1) EPA believes this to be customary industry practice, although it recognizes some laboratories will explicitly cost animal space.

2) EPA estimates that the cost for animal space will be small relative to the total costs of the standards. If one assumes about 100 square feet for 400 mice, 250 square feet for 400-500 rats, and 700 square feet for 48 dogs, and a cost of $6/square foot/year, the costs are $1,200, $3,000 and $8,400 respectively for a two year test.

4. Items Excluded From Estimates

The cost of a number of items have been excluded from these cost estimates. The items and the rationale for exclusion are explained below.

a. Transitional Costs

The cost estimates represent only the marginal cost of an additional chronic effects test in a laboratory, and not the transitional costs that will be incurred the first few times a laboratory conducts tests in accordance with these standards. These transitional costs, e.g., design of a protocol, establishment of a quality assurance unit, and development of computer programs for statistical analysis, will be incurred in varying degrees from laboratory to laboratory depending on current practices and compliance with FDA Good Laboratory Practice standards.

b. Regulatory Liaison

No estimate is provided for the costs of regulatory liaison with EPA. The extent of these costs will depend on the degree of variance from the EPA standard of the submitted protocol. The informal nature of the mechanisms for handling submitted study plans should minimize these costs.

c. Chemical Characterization

The required chemical characterization for chronic effects testing would in almost every case have been performed prior to short term testing of the chemical and would not be done again prior to chronic health effects testing. Therefore, no cost is included in these estimates for chemical characterization.

d. Ancillary Studies

The cost of pharmacokinetic and metabolic studies are not included in the estimates because they are optional studies undertaken at the discretion of the sponsor.

e. Stability Studies, Contaminant Analysis, Other Studies

Section 772.113 requires that certain chemical tests be performed to determine:

1) the stability of the test substance in the test mixture;
2) methods to assure homogeneous mixing of the test substance in the test mixture (feed or other carrier material);
3) concentration of test substance in each test mixture;
4) contaminants in the feed or vehicle; and
5) nutrient content of feed.

The costs of these tests are expected to vary considerably from chemical to chemical and may be quite expensive. These variations are due to the analytical methodologies required for a particular chemical and the extent to which the analytical methodology needs to be developed. Because of this variability per chemical, EPA may address this item in greater detail when chemicals are selected for testing in Section 4 test rules.

5. Variations In Costs

The actual cost of testing that will be conducted in response to Section 4 testing requirements is likely to vary considerably from the estimates provided here for oncogenic, chronic, and combined testing. There are three reasons for these variations:

1) The cost of conducting exactly the same test will vary from laboratory to laboratory due to differences in labor productivity, salary and overhead rates, equipment, etc.

2) The cost of testing chemicals in the same laboratory will vary depending on a number of factors. These factors include: the number of dose levels, species, route(s) of exposure, extent of pathology conducted, duration of tests, and inclusion of ancillary studies.

3) The cost estimates probably make some estimation errors and oversimplify some factors.

The cost estimates are those to meet the minimum requirements of the standard. The actual cost of testing will be greater to the extent that optional testing is conducted and transitional costs are incurred.

6. Use Of Ranges

While ranges have not been used in the estimates, every number used in the calculations below could undoubtedly be more accurately reflected by a range. While this might initially seem desirable, there are two practical problems in implementing the concept. First, the information requirements and number of calculations of the costing methodology used here are already substantial; use of ranges would increase these. Second, the appropriate range estimate for subparts of the standard or for the entire standard would be difficult to determine. This is true because:

1) Range estimates almost always have an implicit or explicit confidence level associated with them -- e.g., 95% of the time the number will fall within the range.

2) When range estimates with confidence levels are added together, the range for the sum with the same confidence level (e.g. 95%) is more narrow than that obtained by adding all the low ends of the ranges for one estimate and the high ends for the other estimate. The range estimate produced by all the low ends and high ends would be too broad to be meaningful.

For these reasons, the appropriate range estimates are very difficult to determine.

C. FIXED COSTS

1. Summary

The estimates assume that the following testing costs do not vary with the size of the experiment or the type of test (oncogenic, chronic, or combined):

Protocol Design and Study Plan Submission	$ 8,300
Project Management and Preparation of Final Report	$20,000
Statistical Analysis	$18,200
	$46,500, or approximately $45,000

Clearly, this is somewhat of an oversimplication -- these costs will be greater for a combined study than for either an oncogenic or chronic study, and may differ somewhat with the size of the experiment; however, the differences are not expected to be significant.

The rule requirements, assumptions, and calculations for these items are explained below.

2. Protocol Design and Study Submission

Rule Requirements: The rule requirements for study plan submission are stated in detail in Good Laboratory Practices Subpart B, Section 772.110-1(g)(1) and in the General Chronic Health Effects Standards, Subpart D, Section 772.113-1(f).

Assumptions: The following assumptions were used in calculating the cost of protocol design and study submission:

- Most of the effort in protocol design is estimated to be a one-time transitional cost, i.e., a cost incurred the first time a laboratory conducts a test in accordance with these standards. The cost to modify this previously developed protocol to apply to the particular chemical at issue should be relatively minor, approximately one person-month of senior personnel effort. It is the latter cost which is estimated here.

- Salary of senior personnel required is approximately $50,000. This represents an approximate average of those personnel required (study director, senior statistician, pathologist, etc.).

Cost:
1/12 person-year x ($50,000 x 100% overhead) = $8,333 or approximately $8,300

3. Project Management and Preparation of Final Report

Rule Requirements: The explicit requirements in the standards for project management are contained in the Good Laboratory Practice Standards, Subpart B, Section 772.110-1 (c)(2), (3), and (4). This includes requirements for testing facilities management, a study director, and a quality assurance unit. The reporting requirements for the final report are specified in the general requirements for chronic health effects studies contained in Subpart D, Section 772.113-1(j). The Agency plans to propose specific standards for data formatting later this year.

Assumptions: The following assumptions were used in calculating the cost of project management and preparation of final report:

- Transitional costs (e.g., establishment of a quality assurance unit or adoption of new reporting requirements) are not included in the estimates.
- A precise estimate of report costs can not be made at this time. The analysis of data formatting procedures to be conducted later this year should provide a basis for estimating these reporting costs.

Cost: A preliminary estimate is that the costs of project management and reporting will be approximately $20,000. EPA solicits comment on how this cost estimate might be refined.

4. Statistical Analysis

Rule Requirements: Information specified in Subpart D, Section 772.113-1(j)(2)(ii) must be reported for each test group. In many cases, this will require the computation of medians, means, standard deviations, and other statistical measures.

Assumptions: The following assumptions were used in calculating the cost of statistical analysis:

- Computerized statistical programs will be the least costly way of performing this analysis. Computer costs are expected to be minimal, once the statistical programs are operational on the computer. The costs to develop these programs are transitional costs and are not included in the estimates.

- The approximate staff time requirements are as follows:

senior statistician	1 person-month
intermediate statistician	1 person-month
junior analysts	3 person-months

- The salary rates are as follows:

senior statistician	$40,000
intermediate statistician	$24,000
junior analysts	$15,000

Cost:

1/12 person-year x ($40,000/yr x 100% overhead) = $ 6,666
1/12 person-year x ($24,000/yr x 100% overhead) = 4,000
3/12 person-year x ($15,000/yr x 100% overhead) = 7,500
Total $18,166 or approximately $18,200

D. ONCOGENIC EFFECTS

1. Summary

The estimated costs of the oncogenic effects test standards are shown below in Table 2.

Table 2
Costs of Oncogenic Effects Test Standards

	Mouse	Rat	Total
Fixed Costs:	-	-	$ 45,000 1/
Variable Costs:			
Animal Procurement	$ 1,100	$ 1,400	$ 2,500
Rations	300	1,000	1,300
Animal Care	32,800	63,500	96,300
Clinical Laboratory Tests	1,300	1,300	2,600
Microscopic Examinations	45,000	45,000	90,000
Necropsy and Histological Preparation	75,000	75,000	150,000
Total Variable Costs	$155,500	$187,200	$342,700
Total Oncogenic Costs	-	-	$387,700
Prechronic Testing Costs:	-	-	50,900
Total Costs Including Prechronic			$438,600

These estimates assume testing in 400 rats and 400 mice. This assumes testing at 3 different dose levels plus a control group, 50 animals in each group and testing of both sexes -- (3 dose levels + 1 control group) x 50 animals x 2 sexes = 4 x 50 x 2 = 400 animals of each species.

These estimates assume that the duration of the test is 2 years. In the cost calculations below, the duration factor will be expressed as 2 years, 104 weeks, or 730 days, depending on how other assumptions are expressed.

The details of how the variable costs of oncogenic testing and associated prechronic testing were calculated are provided below.

1/ See Section C, "Fixed Costs" for details.

2. Variable Costs

Variable costs of oncogenic testing include the costs of animal procurement, rations, animal care, clinical laboratory tests, microscopic examinations, and necropsy and histological preparation. Each of these costs are calculated separately, and separate estimates are presented for mice and rats. These calculations are discussed below.

a. Animal Procurement

Rule Requirement: EPA does not stipulate that a specific strain or stock of rats or mice must be used, nor does EPA express a preference among inbred, outbred, and hybrid strains. However, the rule does require that test animals be from established strains and/or stocks. As part of the study plan submission, the sponsor must provide historical data on the lifespan and types and incidences of disease for the selected strains.

Assumptions: The following assumptions were used in calculating the cost of animal procurement:

- Mice cost $2.35 each;
- Rats cost $2.85 each;
- 480 rats and 480 mice will be purchased.

It is assumed that 20% more rats and mice will be purchased than started on the test so that testing can begin on healthy animals. (See Section B, page III-8).

Cost: Animal procurement is estimated to cost $2.500, calculated as follows:

480 mice x $2.35/mouse =	$1,130
480 rats x $2.85/rat =	$1,370
	$2,500

b. Rations

Rule Requirement: A standardized rodent diet containing specified nutrient levels and produced from certain feed stocks or ingredients (see Appendix A, Subpart D, Section 772.113-1) is required unless information is provided by the sponsor to justify deviation. Diets for species other than the rodent must be approved by EPA.

Assumptions: The following assumptions were used in calculating the cost of rations:

- Mice consume 4.5 gm of food per day;
- Rats consume 13.5 gm of food per day;
- Rations cost $235/ton. 1/

Cost: Rations are estimated to cost $1,360, calculated as follows:

400 rats x 13.5 gm/day x 730 days = 3,942 kg.	
3,942 kg. x .0011 tons/kg. x $235/ton =	$1,020
400 mice x 4.5 gm/day x 730 days = 1,314 kg.	
1,314 kg. x .0011 tons/kg. x $235/ton =	$ 340
	$1,360 or approximately $1,300

c. Animal Care

Animal care consists of clinical examination, observation, feeding, and cage cleaning.

i. Clinical Examination

Rule Requirements: Clinical examination, including the weighing of each animal, must be conducted at least once a week during the first 13 weeks, and every two weeks thereafter. Clinical examination must include observation relating to food and water consumption, morbidity, mortality and causes thereof, pharmacologic effects, and behavioral changes.

Assumptions: The following assumptions were used in calculating the cost of clinical examinations.

- A technical employee can weigh and examine 25 animals per hour;
- The salary of the technical employee is $12,000 per year.

Cost: Clinical examination is estimated to cost $21,600, calculated as follows:

1/ Low bid to supply NIH-31 rodent diet containing 18% crude protein to NIH for 12 months was $235.20 per ton. (Letter from Joseph Knapka, NIH, to Carl Morris, EPA dated January 11, 1979).

Mice:

400 animals ÷ 25 animals/hour = 16 hours/week

Weekly exams for 13 weeks:	
13 weeks x 16 hours/week	208 hours
Biweekly exams for 91 weeks	
(91 weeks ÷ 2) x 16 hours/week	728 hours
	936 hours

$$\frac{936\text{ hours}}{2{,}080\text{ hours/year}} = .45\text{ person-years}$$

. 45 person-years x ($12,000/yr. x 100% overhead) =	$10,800

Rats:

The above calculation applies to rats as well - i.e., the cost is:	$10,800
Total	$21,600

ii. Observation

Rule Requirement: A technical employee must observe the test animals every 12 hours throughout the test period.

Assumptions: The following assumptions were made in calculating the cost of observation:

- The time requirements for observation are assumed to be as follows:
  - For the first 18 months of the study, 1 hour per day for 400 mice and 3 hours per day for 400 rats;
  - For the last 6 months of the study where the health status of more animals will be problematical, 3 hours per day for 400 mice and 5 hours per day for 400 rats.
- The salary of the technical employee is $12,000/yr.

Cost: The estimated cost of observation is $42,200, calculated as follows:

Mice:

First 18 months:	
(365 days x 1.5 years) x 1 hour/day =	547.5 hours
Last 6 months:	
(365 days x .5 years) x 3 hours/day =	547.5 hours
	1095 hours

$$\frac{1{,}095 \text{ hours}}{2{,}080 \text{ hours/year}} = .53 \text{ person-years}$$

.53 person-years x ($12,000/yr. x 100% overhead) = $12,720 or approximately $12,700.

Rats:

First 18 months:

(365 days x 1.5 years) x 3 hours/day = 1,642.5 hours

Last 6 months:
(365 days x .5 years) x 5 hours/day = 912.5 hours

2,555 hours

$$\frac{2{,}555 \text{ hours}}{2{,}080 \text{ hours/year}} = 1.23 \text{ person-years}$$

1.23 person-years x ($12,000/yr. x 100% overhead) = $29,520 or approximately $29,500.

Total $42,200

iii. Feeding

Rule Requirement: For a feeding study, the test substance must be administered *ad libitum* (continuously available).

Assumptions: The following assumptions were made in calculating the cost of feeding:

- 1 cage will contain 5 mice and
  1 cage will contain 2 rats;
- A technician can process 30 cages/hour for feeding the test animals;
- The technician's salary is $8,000/year;
- The feed containers for mice and rats will be refilled once every third day.

Cost: The estimated cost of feeding is $17,500, calculated as follows:

Mice:

400 mice ÷ 5 mice/cage = 80 cages
80 cages ÷ 30 cages/hour = 2.67 hours/feeding.

2.67 hours/feeding x (730 days x 1/3) = 650 hours

$$\frac{650 \text{ hours}}{2{,}080 \text{ hours/year}} = .31 \text{ person-years}$$

.31 person-years x ($8,000/yr. x 100% overhead) = $4,998 or approximately $5,000.

Rats:

400 rats ÷ 2 rats/cage = 200 cages
200 cages ÷ 30 cages/hour = 6.67 hours/feeding

6.67 hours/feeding x (730 days x 1/3) = 1,623 hours

$$\frac{1{,}623 \text{ hours}}{2{,}080 \text{ hours/year}} = .78 \text{ person-years}$$

.78 person-years x ($8,000 x 100% overhead) = $12,480 or approximately $12,500

Total $17,500

iv. Cage Cleaning

Rule Requirement: The recommendations of HEW Publication No. (NIH) 74-23, "Guide for the Care and Use of Laboratory Animals" apply except where standards are specified in the Animal Welfare Act of 1970 (9CFR Part 3). HEW Publication No. 74-23 states that for routine maintenance of small rodents, one to three changes per week of litter or bedding should suffice.

Assumptions: The following assumptions were made in calculating the cost of cage cleaning:

- 1 cage will contain 5 mice;
- 1 cage will contain 2 rats;
- Cage cleaning will occur twice a week (average of the one to three changes per week cited above);
- A technician can clean 30 cages per hour;
- The technician's salary is $8,000/year.

Cost: The estimated cost of cage cleaning is $15,000, calculated as follows:

Mice:

400 mice ÷ 5 mice/cage = 80 cages

80 cages ÷ 30 cages/hour = 2.67 hours/cage cleaning

2.67 hours/cage cleaning x (104 weeks x 2 times) = 555 hours

$\frac{\text{555 hours}}{\text{2,080 hours/year}}$ = (.27 person-years)

.27 person-years x ($8,000/yr. 100% overhead) = $4,320 or approximately $4,300

Rats:

400 rats ÷ 2 rats/cage = 200 cages

200 cages ÷ 30 cages/hour = 6.67 hours/cage cleaning

6.67 hours/cage cleaning x (104 weeks x 2) = 1,387 hours

$\frac{\text{1,387 hours}}{\text{2,080 hours/year}}$ = .67 person-years

.67 person-years x ($8,000 x 100% overhead) = $10,720 or approximately $10,700

Total $15,000

v. Summary Chart

Summarizing the above costs, the estimated costs of animal care are as follows:

	Mouse	Rat	Total
Clinical Examination	$10,800	$10,800	$21,600
Observation	12,700	29,500	42,200
Feeding	5,000	12,500	17,500
Cage Cleaning	4,300	10,700	15,000
Total Animal Care Costs	$32,800	$63,500	$96,300

d. Clinical Laboratory Tests

Rule Requirements: The following quantitative hematologic determinations are required twice during the study on a minimum of 8 predesignated animals per group: hematocrit, hemoglobin, erythrocyte count, total and differential leukocyte counts, platelet count, and prothrombin and clotting time. If hematologic evidence of anemia is present at one year, reticulocyte counts must be performed within one week of the determination.

Assumptions: The following assumptions were made in calculating the cost of clinical laboratory tests:

- The cost of a hematologic determination is $10/animal;
- 8 animals will be tested per group (this is the minimum requirement of the standard).

Cost: The estimated cost of clinical laboratory examination is $2,600, calculated as follows:

Mice:

8 animals/group x 8 groups = 64 animals		
64 animals x 2 times = 128 tests		
128 tests x $10/test =		$1,280

Rats:

The above calculation applies to rats as well -- i.e., the cost is:		$1,280
	Total	$2,560 or approximately $2,600

e. Microscopic Examinations

Rule Requirements: Microscopic examination must be performed on all tissues described in Subsections (b)(2)(ii), (b)(2)(v), and (b)(2)(vi) of Section 772.113-2. Subsection (b)(2)(v) requires that when there is clinical evidence of specific toxicologic or pharmacologic effects related to specific target organs, the necropsy and microscopic examinations of these target organs must be conducted in greater detail. Subsection (b)(2)(vi) requires that sections of tissues from a minimum of ten animals from each test group and in all animals in which clinical or grossly observable evidence of disease is present be examined microscopically. If microscopic examination reveals evidence of disease in any of the tissues, then these target tissues must be examined microscopically in all animals.

Assumptions: The following assumptions were used in calculating the cost of microscopic examinations:

- The routine microscopic examination of Subsection (b)(2)(ii) represents approximately 50 sections per animal.

- The additional examinations required by Subsection (b)(2)(v) represents approximately a 10% increase over the routine requirements, or 5 additional sections,
- The special examinations of Subsection (b)(2)(vi) represent only 1-2 sections. These 1-2 sections will be examined in anywhere from 20-100% of the animals. This requirement should represent, on average, no more than 1 section per test animal,
- Total sections required per animal are therefore as follows:

routine:	50
additional:	5
special:	1
	56 sections.

- A pathologist can read slides from 50,000 sections per year. This figure is from an NCI survey which assumed four hours a day at the microscope. This figure has been used in the literature to calculate pathology workload [1]
- The salary of the pathologist is $50,000 per year.

Cost: Microscopic examination is estimated to cost $90,000, calculated as follows:

Mice:

400 animals x 56 sections/animal = 22,400 sections

$$\frac{22{,}400 \text{ sections}}{50{,}000 \text{ sections/year}} = .45 \text{ person-years}$$

.45 person-years x ($50,000/year x 100% overhead) = $45,000

Rats:

The above calculation applies to rats as well-- i.e., the cost is: $45,000

$90,000

f. Necropsy and Histological Preparation

Rule Requirements: The rule requirements are stated in detail in the standards. The requirements provide for:

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[1] Page, Norbert "Chronic Toxicity and Carcinogenicity Guidelines" Journal of Environmental Pathology and Toxicology, Vol. 1, pp. 161-182, 1977.

- an initial examination of the external surfaces and all orifices followed by an internal examination of tissues and organs *in situ*;
- inflation of urinary bladder and lungs;
- trimming specifications;
- multiple sections (step cuts) must be made on each tissue or organ that contains gross evidence of a neoplasm or lesion and on each tissue or organ in which a metastasis may be anticipated.

Assumptions: The following assumptions were used in calculating the cost of necropsy and histological preparation:

- 13 hours/animal of technical time are required for gross necropsy and examination, preparation of tissues, and staining of slides;
- The salary of the necropsy technical employee is $15,000 per year.

Cost: Necropsy and histological preparation is estimated to cost $150,000, calculated as follows:

Mice:

400 animals x 13 hours/animal = 5,200 hours

$\frac{\text{5,200 hours}}{\text{2,080 hours}}$ = 2.5 person-years

2.5 person-years x ($15,000/year x 100% overhead)	= $ 75,000

Rats:

The above calculation applies to rats as well -- i.e., the cost is	$ 75,000
Total	$150,000

3. Prechronic Testing Costs

Rule Requirement: A preliminary toxicology study of at least 90 days must be utilized to predict dose levels. If such a study has been completed previously, it may be submitted for this purpose.

Assumptions: The following assumptions are used in calculating the cost of prechronic testing:

- The prechronic testing procedures of the National Cancer Institute (NCI) will be used. These procedures are essentially those described in "Guidelines for

Carcinogen Bioassay in Small Rodents", National Cancer Institute Carcinogens Technical Report Series, No. 1, February 1976, pp. 11-15. Briefly, they consist of the following studies:

- acute toxicity test: 5 dose levels plus controls, 5 animals/ sex/dose level, 2 species, 14-day observation period following single dosing, and a 16-day quarantine period.
- repeated-dose test: 5 dose levels plus controls, 5 animals/ sex/dose level, 2 species, and a 14-day exposure period. No additional observation following exposure period, and a 16-day quarantine period.
- subchronic test: 5 dose levels plus controls, 10 animals/sex/dose level, 2 species, 90-day exposure period, and a 16-day quarantine period. No additional observation following exposure period. Pathological examination of 32 tissues in control group and high dose group. Target tissues thus identified are then examined in next-to-highest dose group and proceeding through lower dose groups until no effects are found in the particular tissue.

- The cost of these prechronic procedures will be the same as currently estimated by NCI. NCI estimates that their feeding studies cost approximately $268,000 and that prechronic test costs constitute approximately 19% of this total. 1/

NOTE: The fact that prechronic studies are assumed for costing purposes does not indicate that EPA is thereby providing guidance as to appropriate prechronic testing procedures.

Cost: Prechronic studies are estimated to cost $50,900, calculated as follows:

19% of total cost of $268,000 = $50,920, or approximately $50,900.

1/ Personal communication with Donald Minnick, Tracor Jitco, April 20, 1979. Tracor Jitco is the prime contractor for NCI's Carcinogen Bioassay Program.

E. CHRONIC EFFECTS

1. Summary

The estimated costs of the chronic effects standards are shown below in Table 3.

Table 3
Cost of Chronic Effects Test Standards

	Rat	Dog	Total
Fixed Costs:	-	-	$45,000 1/
Variable Costs:			
Animal Procurement	$ 1,600	$ 8,700	$ 10,300
Rations	1,500	- 2/	1,500
Animal Care	95,600	175,200 2/	270,800
Clinical Laboratory Tests	9,600 3/	11,500	21,100
Microscopic Examination	62,000	13,000	75,000
Necropsy and Histological Preparation	87,000	18,000	105,000
Total Variable Costs	$257,300	$226,400	$483,700
Total Chronic Costs	-	-	$528,700
Prechronic Costs:			$100,500
Total Costs Including Prechronic			$629,200

The estimates assume testing in 464 rats and 48 dogs. This assumes testing at 3 different dose levels plus a control group, and testing in both sexes--(3 dose levels + 1 control group) x 2 sexes = 4 x 2 = 8 groups in each species. Group size in the rat is 58--this consists of 50 per group as in the oncogenic study plus an additional 8 animals required for clinical laboratory tests. Group size for the dog is 6--the clinical laboratory tests are performed on all dogs and no additional animals are required for this purpose.

Rat: 8 groups x 58 rats/group = 464 rats
Dog: 8 groups x 6 dogs/group = 48 dogs.

The details of how the variable costs of chronic testing and associated prechronic testing were calculated are provided below.

1/ See Section C, "Fixed Costs" for detailed discussion.

2/ Cost of rations for dog is included in animal care.

3/ This represents the costs of the tests themselves. The addition of 8 rats per group costs an additional $34,200 which appears in this table under animal procurement, rations, etc. The true cost of these tests in the rat is therefore approximately $43,800.

2. Variable Costs

Variable costs of chronic testing include the costs of animal procurement, rations, animal care, clinical laboratory tests, microscopic examinations, and necropsy and histological preparation. Each of these costs were calculated separately for the rat; for the dog, the costs of rations and animal care were developed as a single estimate. These calculations are discussed below.

a. Animal Procurement

Rule Requirement: EPA does not stipulate that a specific strain or stock must be used, nor does EPA express a preference among inbred, outbred, and hybred strains. However, test animals must be from established strains and/or stocks. As part of the study plan submission, the sponsor must provide historical data on the lifespan and types and incidence of disease for the strains.

Assumptions: The following assumptions were used in calculating the cost of animal procurement:

- Rats cost $2.85 each;
- Dogs cost $150 each; 1/
- 560 rats and 58 dogs will be purchased. 2/

Cost: Animal procurement is estimated to cost $10,300, calculated as follows:

560 rats x $2.85/rat =	$ 1,600
58 dogs x $150/dog =	$ 8,700
	$10,300

b. Rations (rat only)

Rule Requirement: A standardized rodent diet containing specified nutrient levels and produced from certain feed stocks or ingredients (see Appendix A, Subpart D, Section 772.113-1) is required unless

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1/ Two estimates were obtained here: $300/beagle from a non-profit testing laboratory and $150/beagle from a commercial testing laboratory. The latter estimate was for a 7-8 month old dog which would be more expensive than the dogs used for the test standards, where dosing begins by 10 weeks and purchase (to allow for acclimatization period) would occur even earlier. EPA believes $150/dog is a conservative and reasonable figure.

2/ It is assumed that 20% more rats and dogs will be purchased than started on the test so that testing can begin on healthy animals. (See Section B, page III - 8).

information is provided by the sponsor to justify deviation. Diets for species other than the rodent must be approved by EPA.

Assumptions: The following assumptions were used in calculating the cost of rations:

- Rats consume 13.5 gm of food per day;
- Rations cost $235/ton; 1/
- Duration of the test is 2 1/2 years or 913 days (see Section B, page III - 7).

Cost: Rations are estimated to cost $1,500, calculated as follows:

464 rats x 13.5 gm/day x 913 days = 5,719 kg.
5,719 kg. x .0011 tons/kg. x $235/ton =
$1,478 or
approximately $1,500

c. Animal Care (rat only)

Animal care consists of clinical examination, observation, feeding, and cage cleaning.

i. Clinical Examination

Rule Requirements: Clinical examination, including the weighing of each animal, must be conducted at least once a week during the first 13 weeks, and every two weeks thereafter. Clinical examination must include observation relating to food and water consumption, morbidity, mortality and causes thereof, pharmacologic effects, and behavioral changes.

Assumptions: The following assumptions were used in calculating the cost of clinical examinations:

- A technical employee can weigh and examine 25 animals per hour;
- The salary of the technical employee is $12,000 per year.
- Duration of test is 2 1/2 years or 130 weeks. (See Section B, page III - 7).

1/ Low bid to supply NIH-31 rodent diet containing 18% crude protein to NIH for 12 months was $235.20 per ton. (Letter from Joseph Knapka, NIH, to Carl Morris, EPA dated January 11, 1979).

Cost: Clinical examination is estimated to cost $15,400, calculated as follows:

464 animals ÷ 25 animals/hour = 18.6 hours/week

Weekly exams for 13 weeks: 13 weeks x 18.6 hours/week	242 hours
Biweekly exams for 117 weeks: (117 weeks ÷ 2) x 18.6 hours/week	1088 hours
	1330 hours

$\frac{1330 \text{ hours}}{2080 \text{ hours/year}}$ = .64 person-years

.64 person-years x ($12,000/yr. x 100% overhead) = $15,360 or approximately $15,400

ii. Observation

Rule Requirement: A technical employee must observe the test animals every 12 hours throughout the test period.

Assumptions: The following assumptions were made in calculating the cost of observation:

- The time requirements for the two daily observations are assumed to be as follows:
  - For the first 18 months of the study, 3.5 hours per day for 464 rats;
  - For the last 12 months of the study where the health status of more animals will be problematical, 5.8 hours per day for 464 rats.
- The salary of the technical employee is $12,000/yr.
- Duration of the test is 2 1/2 years.

Cost: The estimated cost of observation is $46,600, calculated as follows:

First 18 months: (365 days x 1.5 years) x 3.5 hours/day =	1,916 hours
Last 12 months: (365 days x 1.0 years) x 5.8 hours/day =	2,117 hours
	4,033 hours

$\frac{4{,}033 \text{ hours}}{2{,}080 \text{ hours/year}}$ = 1.94 person-years

1.94 person-years x ($12,000 x 100% overhead) =	$46,560 or approximately $46,600

iii. Feeding

Rule Requirement: For a feeding study, the test substance must be administered *ad libitum* (continuously available).

Assumptions: The following assumptions were made in calculating the cost of feeding:

- 1 cage will contain 2 rats;
- A technician can process 30 cages/hour for feeding the test animals;
- The technician's salary is $8,000/year;
- The feed containers for rats will be refilled once every third day;
- Duration of test is 2.5 years or 913 days. (See Section B, page III - 7).

Cost: The estimated cost of feeding is $18,100, calculated as follows:

464 rats ÷ 2 rats/cage = 232 cages
232 cages ÷ 30 cages/hour = 7.73 hours/feeding

7.73 hours/feeding x (913 days x 1/3) = 2,352 hours

$$\frac{2{,}352 \text{ hours}}{2{,}080 \text{ hours/year}} = 1.13 \text{ person-years}$$

1.13 person-years x ($8,000/year x 100% overhead) =	$18,080 or approximately $18,100

iv. Cage Cleaning

Rule Requirement: The recommendations of HEW (NIH) Publication No. 74-23, "Guide for the Care and Use of Laboratory Animals" apply except where standards are specified in the Animal Welfare Act of 1970 (9CFR Part 3). HEW Publication No. 74-23 states that for routine maintenance of small rodents, one to three changes per week of litter or bedding should suffice.

Assumptions: The following assumptions were made in calculating the cost of cage cleaning:

- 1 cage will contain 2 rats;
- Cage cleaning will occur twice a week (average of the one to three changes per week cited above);
- A technician can clean 30 cages per hour;
- The technician's salary is $8,000/year;
- Duration of test is 2.5 years or 130 weeks (see Section B, page III - 7).

Cost: The estimated cost of cage cleaning is $15,500, calculated as follows:

464 rats ÷ 2 rats/cage = 232 cages
232 cages ÷ 30 cages/hour = 7.73 hours/cage cleaning

7.73 hours/cage cleaning x (130 weeks x 2) = 2,010 hours

$$\frac{2{,}010 \text{ hours}}{2{,}080 \text{ hours/year}} = .97 \text{ person-years}$$

.97 person-years x ($8,000/yr. x 100% overhead) = $15,520 or approximately $15,500

v. Summary Chart

Summarizing the above costs, the estimated costs of animal care for rats are as follows:

	Rat
Clinical Examination	15,400
Observation	46,600
Feeding	18,100
Cage Cleaning	15,500
	$95,600

d. Rations and Animal Care (dog)

Rule Requirements: The requirements for dogs are as specified in the rations and animal care sections above.

Assumptions: The following assumptions were made in calculating the cost of rations and animal care for the dog.

- Duration of the test is 2 years or 730 days (see Section B, page III - 7).
- Dogs will receive very good animal care because each dog represents a sizable investment of resources.

- Rations and animal care for dogs will cost $5/dog/day. 1/

Cost: Rations and animal care for the dog is estimated to cost $175,200, calculated as follows:

$5/dog/day x 48 dogs x 730 days = $175,200

e. Clinical Laboratory Tests

Rule Requirements: A number of determinations are required on a minimum of eight rodents in each group and on all non-rodents. These determinations are specified in detail in Subpart D, Section 772.113-3(b)(1)(ii). Briefly summarizing, hematology, blood chemistry, urinalysis, function tests, and residue analysis are required at least at 3,6,12,18, and 24 months and at study termination. Function tests are also required at the beginning for non-rodents.

Assumptions: The following assumptions were made in calculating the cost of clinical laboratory tests:

- These determinations will be performed each time in 8 rats per group and 6 non-rodents per group. 8 rats per group represents the minimum requirement of the standard whereas 6 non-rodents per group results from using 6 non-rodents per group for chronic testing, which is the minimum requirement for the entire chronic test.

- The cost of these determinations is $25/rat and $40/dog each time the set of determinations is performed.

- Hematology, blood chemistry, urinalysis, and residue analysis will be performed 5 times, function tests will be performed 6 times in the non-rodents. All analyses will be performed 6 times for the rodent. The non-rodent requirements differ from the rodent requirements because the minimum duration periods differ between the two species. In the non-rodent, all tests except the function test will only be performed 5 times in the 2 year test period. The function tests in the non-rodent are required at the beginning of the study and thus must be performed a minimum of six times.

1/ EPA assumption based on limited industry data.

- Costs estimates were derived based on all tests being performed 6 times since the error will be small relative to other estimation errors.

Cost: Clinical laboratory tests are estimated to cost $21,100, calculated as follows:

Rat:	
6 times x 8 rodents/group x 8 groups x $25/rat/time =	$ 9,600
Dog:	
6 times x 6 dogs/group x 8 groups x $40/time =	$11,520
Total	$21,120
	or approximately $21,100.

f. Microscopic Examinations

Rule Requirements: Microscopic examination must be performed on all tissues described in Subsections (b)(2)(ii), (b)(2)(v), and (b)(2)(vi) of Section 772.113-3. Subsection (b)(2)(v) requires that when there is clinical evidence of specific toxicologic or pharmacologic effects related to specific target organs, the necropsy and microscopic examinations of the suspected target organs must be conducted in greater detail. Subsection (b)(2)(vi) requires that sections from certain tissues be microscopically examined from a minimum of ten animals from each test group and from all animals with clinical or grossly observable evidence of disease. If microscopic examination shows evidence of disease in any of these tissues, then these target tissues must be examined microscopically for all animals.

Assumptions: The following assumptions were used in calculating the cost of microscopic examinations:

- The routine microscopic examination of Subsection (b)(2)(ii) represents approximately 60 sections per animal.
- The additional examinations of Subsection (b)(2)(v) represent approximately a 10% increase over the routine requirements, or 6 sections per animal.
- The special examinations of Subsection (b)(2)(vi) represent only 1-2 sections. These 1-2 sections will be examined in anywhere from 20-100% of the animals. This requirement should represent, on average, no more than 1 section per test animal.

- Total sections required per animal are therefore as follows:

routine: 60
additional: 6
special: 1
67 sections.

- A pathologist can read slides from 50,000 sections per year for the rat. This figure is from an NCI survey which assumed four hours a day at the microscope. This figure has been used elsewhere to calculate pathology workload. 1/
- A pathologist can read slides from 25,000 sections per year for the dog. 2/
- The salary of the pathologist is $50,000 per year.

Cost: Microscopic examination is estimated to cost $75,000, calculated as follows:

Rats:

464 rats x 67 sections/animal = 31,088 sections

$\frac{31,088 \text{ sections}}{50,000 \text{ sections/year}}$ = .62 person-years

.62 person-years x ($50,000/year x 100% overhead) = $62,000

Dogs:

48 dogs x 67 sections/animal = 3,216 sections

$\frac{3,216 \text{ sections}}{25,000 \text{ sections/year}}$ = .13 person-years

.13 person-year x ($50,000/year x 100% overhead) = $13,000

Total $75,000

1/ Page, Norbert "Chronic Toxicity and Carcinogenicity Guidelines" Journal of Environmental Pathology and Toxicology, Vol. 1, pp. 161-182, 1977.

2/ EPA assumption, based on an extrapolation from the 50,000 figure for the rat.

g. Necropsy and Histological Preparation

Rule Requirements: The rule requirements are stated in detail in the standards (Section 772.113-3(b)(2)). The requirements provide for:

- an initial examination of the external surfaces and all orifices followed by an internal examination of tissues and organs in situ;
- inflation of urinary bladder and lungs;
- trimming specifications; and
- multiple sections (step cuts) on each tissue or organ that contains gross evidence of a neoplasm or lesion and on each tissue or organ in which a metastasis may be anticipated.

Assumptions: The following assumptions were used in calculating the cost of necropsy and histological preparation.

- 13 hours/rat and 26 hours/dog 1/ of technical employee time are required for gross necropsy and examination, preparation of tissues, and staining of slides.

- The salary of the necropsy technical employee is $15,000 per year.

Cost: Necropsy and histological preparation is estimated to cost $105,000, calculated as follows:

Rats:

464 animals x 13 hours/animal = 6,032 hours

$\frac{\text{6,032 hours}}{\text{2,080 hours/year}}$ = 2.9 person-years

2.9 person-years x ($15,000/year x 100% overhead) =	$87,000

1/ 26 hours/dog is an extrapolation from the 13 hours/rat figure.

Dogs:

48 animals x 26 hours/animal = 1,248 hours

$\frac{1,248 \text{ hours}}{2,080 \text{ hours/year}}$ = .6 person-years

.6 person-years x ($15,000/year x 100%
overhead) = $18,000

Total 105,000

3. Prechronic Costs

Rule Requirement: A preliminary toxicology study of at least 90 days must be utilized to predict dose levels. If such a study has been completed previously, it may be submitted for this purpose.

Assumptions: The following assumptions are used in calculating the cost of prechronic testing.

- The following testing will be assumed:
  - rats: National Cancer Institute (NCI) prechronic testing procedures will be used. These procedures are essentially those described in "Guidelines for Carcinogen Bioassay in Small Rodents", National Cancer Institute Carcinogens Technical Report Series, No. 1, February 1976, pp. 11-15. Briefly, they consist of the following studies:
  - acute toxicity: 5 dose levels plus controls, 5 animals/sex/dose level, 2 species, 14-day observation period following single dosing, and a 16-day quarantine period.

    repeated dose: 5 dose levels plus controls, 5 animals/sex/dose level, 2 species, and a 14-day exposure period. No additional observation following the exposure period and a 16-day quarantine period.

    subchronic: 5 dose levels plus controls, 10 animals/ sex/dose level, 2 species, 90-day exposure period, and a 16-day quarantine period. No additional observation following the exposure period. Pathological examination of 32 tissues in the control group and high dose group. Target tissues thus identified are examined in next-to-highest dose group and proceeding through lower dose groups until no effects are found in the particular tissue.

- Dogs: The same number of dose levels and dogs per group are used as in the long-term study; therefore, 48 dogs will be used.

- subchronic: 3 dose levels plus controls, 6 dogs/sex/dose level, a 90-day exposure period, and no additional observation following the exposure period. Hematology, blood chemistry, and urinalysis on all dogs, three times during the study. Pathological examination of same tissues as in the chronic study.

. Cost of the NCI prechronic study for the rat is only one half of the cost of the study for both rats and mice.

. Cost of the prechronic dog study can be calculated using the same assumptions as in the long-term study. The fixed costs for a prechronic dog study are assumed to be $10,000.

Cost: Prechronic testing is estimated to cost $100,500, calculated as follows:

Rat: $50,900 1/ x 1/2 =	$25,450, or approximately $25,500.
Dog:	
Animal Procurement:	
48 dogs x $150/dog 2/ =	$ 7,200
Rations and Animal Care:	
48 dogs x $5/dog/day 3/ x 90 days =	$21,600

1/ Cost of NCI prechronic testing for rats and mice is 19% of cost of long term NCI test ($268,000), or $50,900 (see Section D-3, page III - 27).

2/ See Section E - 2, pg. III - 29.

3/ See Section E - 2, pg. III - 34.

Clinical Chemistry:

48 dogs x 3 times x $40/set 1/ of observations = $5,760

Microscopic Examination:

48 dogs x 60 sections/dog 2/ = 2,880 sections

$\frac{2,880}{25,000}$ sections/yr. = .12 person-years

.12 person-years x ($50,000/yr. x 100% overhead) = $12,000

Necropsy and Histological Preparation:

48 dogs x 26 hours/dog 3/ = 1,248 hours

$\frac{1,248 \text{ hours}}{2,080 \text{ hours/year}}$ = .6 person-years

.6 person-years x ($15,000/yr. x 100% overhead) = $18,000

Fixed Costs: $10,000

Total Cost $74,560 — $74,560 or approximately $75,000

Total Prechronic Costs for Rat and Dog $100,500

1/ See Section E-2, page III-34.
2/ See Section E-2, page III-36.
3/ See Section E-2, page III-37.

F. COMBINED CHRONIC EFFECTS TEST

1. Summary

The estimated cost of the combined chronic effects standards are shown below in Table 4.

Table 4
Cost of Combined Chronic Effects Test Standards

	Mouse	Rat	Dog	Total
Fixed Costs:	$ -	-	-	$ 45,000 1/
Variable Costs:				
Animal Procurement	$ 1,100	$ 2,200	$ 8,700	$ 12,000
Rations	300	2,100	-	2,400
Animal Care	32,800	131,500	175,200	339,500
Clinical Laboratory Tests	-	13,200	11,500	24,700
Microscopic Examination	53,800	85,300	13,000	152,100
Necropsy and Histological Preparation	75,000	119,600	18,000	212,600
Total Variable Cost	$163,000	$353,900	$226,400	$743,300
Total Combined Chronic Cost				$788,300
Prechronic Costs:				125,900
Total Costs Including Prechronic				$914,200

2. Variable Costs

The figures for the dog in Table 4 are exactly the same as those presented in Table 3, Section E, because the tests are identical. The figures for the mouse are the same as in Table 2, Section D, except for 2 items:

1) Hematology is not required in the combined study for the mouse and thus no cost is shown.
2) Microscopic examination requirements differ slightly. Since the pathology requirements are more extensive in the chronic study than in the oncogenic, the costs are greater in the combined study for the mouse (which uses the chronic requirements) than in the oncogenic. This difference is estimated to amount to: 67 sections (chronic) - 56 (oncogenic) = 11 sections/animal.

1/ See Section C for detailed discussion.

11 sections/mouse x 400 mice = 4,400 sections

$$\frac{4{,}400 \text{ sections}}{50{,}000 \text{ sections/year}} = .088 \text{ person-years}$$

.088 person-years x ($50,000/year x 100% overhead) = $ 8,800

Thus the cost for microscopic examination of the mouse in the combined study is $8,800 greater than in the oncogenic study where the cost is $45,000, or $53,800.

The cost estimates for the rat reflect the fact that 4 or 5 dose levels will be required in the combined chronic tests where only 3 are required in the chronic test. Since EPA is unable to predict what percentage of testing will be requiring at 4 vs. 5 dose levels, equal amounts of testing at both dose levels has been assumed for the purposes of cost estimation. The figures in Table 4 for the rat are 1.375 times the corresponding figures in Table 3. This figure has been calculated as shown below:

5 dose levels (4 dose levels + control) ÷ 4 dose levels (3 dose levels plus control) = 1.25

6 dose levels (5 dose levels + control) ÷ 4 dose levels (3 dose levels plus control) = 1.5
Average = (1.25 + 1.5)/2 = 1.375.

3. Prechronic Testing Costs

The prechronic testing done in the combined test is assumed to be identical to that done in the oncogenic study for the mouse and rat and that done in the chronic study for the dog. Costs are therefore estimated to be approximately $125,900 calculated as follows:

Oncogenic Study - mouse, rat:	$50,900
Chronic Study - dog:	75,000
Total Prechronic Study Costs	$125,900

4. Cost Savings Due To Combined Test

The cost savings due to combining the oncogenic and chronic test standards in the combined test are approximately $125,000 - $150,000. The cost saving from the combined test standard (without prechronic testing) would be approximately $125,000, calculated as follows:

Oncogenic Costs Without Prechronic (Table 2)	$387,700
Chronic Costs Without Prechronic (Table 3)	528,700
Total Cost for Both Tests	$916,400
Combined Costs Without Prechronic (Table 4)	-790,900
Cost Savings	$125,500

The above calculation assumes that no prechronic testing need be performed. If prechronic testing is required for all tests, then there would be an additional cost savings in the combined test of approximately $25,000, calculated as follows:

Oncogenic Prechronic Testing Costs	$ 50,900
Chronic Prechronic Testing Costs	100,500
Total for Both Tests	$151,400
Combined Prechronic Testing Costs	-125,900
Cost Savings	$ 25,500

The cost savings would arise primarily from the fact that when both the oncogenic and chronic tests are performed separately, testing for the rat in 3 dose levels plus controls has to be performed twice. When the combined test is performed, testing for the rat would be at either 4 or 5 dose levels plus controls, and thus there would be a saving of 1-2 dose levels plus a control group over that required when separate tests are performed for oncogenic and chronic effects. Additional savings would result from the fact that the fixed costs for a combined study are lower than the sum of the fixed costs for the oncogenic and chronic studies when conducted separately. Other less significant savings would occur in clinical laboratory tests (approximately $1300), however, these savings are offset slightly by increased microscopic examination requirements ($8800).

G. OTHER DATA ON TESTING COSTS

The National Cancer Institute (NCI) contracts with testing laboratories to perform oncogenicity testing. Cost data from these contracts, when properly adjusted for differences in testing procedures between NCI and EPA, provides a rough check on the cost estimates developed in Sections D, E, and F.

The cost of testing a chemical according to the NCI protocol, as indicated by current cost data from contracts, is as follows: 1/

$268,000 for a feeding study;
$382,000 for gavage; and
$402,000 for an inhalation study.

The NCI cost of a feeding study including prechronic testing ($268,000) can be compared to the EPA estimate ($439,000) by making the following adjustments and then comparing the adjusted total ($382,900) with EPA's estimate ($439,000). The adjustments to NCI's cost are shown below:

	NCI protocol (2 dose levels) 2/	Modified NCI protocol adjusting for differences in number of dose levels, pathology, and other costs
Fixed Costs	$ 81,700	$ 81,700
Variable Costs		
- Pathology	96,500	160,800 3/
- Other	89,800	119,700 4/
Other Costs		
Animal Procurement	-	2,500 5/
Statistical Analysis	-	18,200 6/
Total Cost	$268,000	$382,900

1/ Personal communication with Donald Minnick, Tracor Jitco. Tracor Jitco is the prime contractor for the NCI Carcinogenesis Bioassay Program.

2/ NCI's estimated fixed costs are 30.5% of total cost (includes prechronic, project management, and technical report) and pathology is 36% of total cost (Personal communication with Donald Minnick). Therefore, fixed costs are .305 x $268,000 = $81,740, or approximately $81,700; pathology costs are .36 x $268,000 = $96,480, or approximately $96,500; and other costs are simply the remainder of the $268,000.

3/ EPA estimates that the pathology requirements per animal are approximately 25% greater than those of NCI. EPA also requires 3 dose levels plus a control group whereas NCI requires 2 dose levels. The EPA pathology cost would therefore be 1.25 x 4/3 x $96,500 = $160,833, or approximately $160,800.

No adjustment is made for differences in observation requirements. While EPA requires that a technical employee observe the animals every 12 hours (twice daily), and NCI guidelines require observation only every 24 hours, actual practice of NCI contractors is to observe the animals at the beginning and end of the 9-hour duty-day period (twice daily). Thus, the only cost difference would be that EPA requires either longer shifts, an extra shift, or some overlap, all of which might lead to some increase in cost.

The EPA estimate of $439,000 for a feeding study is thus approximately 15% higher than the adjusted NCI cost of $382,900. The fact that the EPA estimate is within 15% of the adjusted NCI costs is an indication that the costing procedures and assumptions used here are probably reasonable, at least in the aggregate.

4/ Other variable costs are increased proportionately, 4/3 x $89,800 = $119,733, or approximately $119,700. This is due to the difference in the required number of dose levels.

5/ NCI supplies animals for testing so its costs do not include the cost of animal procurement. EPA estimate of $2,500 is used.

6/ NCI does not require statistical analysis. EPA's estimate of $18,200 is used.

H. COST OF ALTERNATIVE STANDARDS

Estimates of the cost of alternative standards are useful in making decisions as to what standards EPA should require and also as an indication of the additional cost that testers will incur for adding optional items, i.e., dose levels, duration, tests, number of animals, etc.

Innumerable variations in the standards could be presented here. Instead, the attempt here has been to focus on items which may be controversial and which appear, based on the estimates, to have a large impact on the cost of the standards. The costs presented in Table 5 on the next page are the marginal cost of varying that item assuming all other parts of the standard are as proposed.

However, the reader is cautioned that the cost for various parts of the protocol are interrelated and cannot always be summed. For example, the cost of adding a dose level is clearly dependent on the extent of the pathology required. Increasing both requirements at the same time would have a greater impact on the cost than the sum of the individual costs. Conversely, decreasing both requirements would have less of a cost saving than the sum of the individual costs.

TABLE 5

Item	Test	Species	Proposed Requirement	Variation	Effect on Cost
Dose Levels	Oncogenic	Mouse	3 dose levels plus control	±1 dose level	±$38,900 [1]
	Oncogenic	Rat	3 dose levels plus control	±1 dose level	±$46,800
	Chronic	Rat	3 dose levels plus control	±1 dose level	±$64,300
	Chronic	Dog	3 dose levels plus control	±1 dose level	±$56,600
	Combined	Mouse	3 dose levels plus control	±1 dose level	±$40,800
	Combined	Dog	3 dose levels plus control	±1 dose level	±$56,600
	Combined	Rat	4 or 5 dose levels plus control	±1 dose level	±$64,300
Duration of Test	Oncogenic	Mouse	24-30 months	±6 months	±$ 8,300 [2]
	Oncogenic	Rat	24-30 months	±6 months	±$16,100
	Chronic	Rat	30 month minimum	±6 months	±$19,400
	Chronic	Dog	24 month minimum	±6 months	±$43,800
	Combined	Mouse	24-30 months	±6 months	±$ 8,300
	Combined	Rat	30 month minimum	±6 months	±$26,700
	Combined	Dog	24 month minimum	±6 months	±$43,800

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[1] EPA assumes that varying the number of dose levels will affect all variable costs proportionately. The cost variation was obtained by dividing the variable costs for each species specified in Tables 2, 3, and 4 by the number of dose levels plus controls (4 for all tests except in the combined test in the rat where the average factor of 4.5 + 1 = 5.5 was used).

[2] EPA assumes that varying the duration of exposure will affect only the cost of rations and animal care. Any indirect effects on pathology due to different age of animals, number of lesions, etc., were assumed to be insignificant. EPA assumes that the number of times clinical laboratory tests are performed is not changed.

TABLE 5 (continued)

Item	Test	Species	Proposed Requirement	Variation	Effect on Cost
Microscopic Examinations	Oncogenic	Mouse	Approximately 56 sections	NCI requirements (approx. 20% less)	- $ 9,000
	Chronic	Rat	Complete microscopic examination in all animals (approximately 67 sections/animal)	Essentially FIFRA proposed guidelines (1) Complete microscopic examination of all animals in high dose level and in control group, and in 15 rats/group in intermediate and low dose groups. (2) For remainder of animals, examination of a small number of tissues plus any target tissues identified from (1) above.	- $ 16,000 [3]

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[3] The cost savings of $16,000 was calculated as follows:

(1) 67 sections/animal x 232 animals = 15,544 sections
(High dose plus control groups --
58 rats/group, 4 groups)

67 sections/animal x 60 animals = 4,020 sections
(15 rats/group in intermediate
and low dose levels, 4 groups)

EPA assumes that the tissues required to be examined plus the target tissues identified from (1) will lead to microscopic examination of approximately 20 sections/animal in the remaining animals, thus

(2) 20 sections/animal x 172 animals = 3,440 sections
(Remainder of animals or
58 - 15 = 43 rats/group, 4 groups)

23,004 sections

Base case = 67 sections x 464 animals = 31,088 sections

Difference = 8,084 fewer sections

$\frac{8,084 \text{ sections}}{50,000 \text{ sections/yr.}}$ = .16 person year; .16 x ($50,000/yr. x 100% overhead) = $16,000

Table 5 (continued)

Item	Test	Species	Proposed Requirement	Variation	Effect on Cost
Clinical Laboratory	Chronic	Rat	8 additional rats per group in addition to the 50.	Essentially FIFRA proposed guidelines: No additional animals (8 are part of 50)	-$34,200 4/

4/ A variable cost savings of $34,200 may be achieved by using 50 rather than 58 rats since fewer animals must undergo other parts of the protocol. This savings was calculated as follows:

Variable Costs (Rat)	257,300	(from Table 3)
Less Clinical Laboratory Tests	9,600	(from Table 3)
	247,700	

$\frac{8}{58}$ x $247,700 = $34,165 cost savings due to use of 50 rats for all cost elements.

IV. Confidentiality Issues

A. Substantiation Policy

As indicated in the Section of the Preamble entitled "Confidentiality and Public Access to Information," EPA is proposing to require persons asserting confidentiality claims for information contained in health and safety studies to provide substantiation for those claims in the form of written answers to specific questions.

EPA's substantiation proposal for health and safety data submitted under Section 4 is based on both policy and legal considerations. First, for each of these types of information there is strong evidence that Congress intended public disclosure of the data to the fullest extent possible. Second, because EPA expects a high volume of requests for disclosure, EPA must have the substantiation readily available in order to reduce the administrative burden of responding to Freedom of Information Act (FOIA) requests. Third, EPA must be prepared to respond to these requests speedily. By including in TSCA such provisions as the Section 4(d)(3) requirement to publish a *Federal Register* notice of receipt of data, Congress evinced a decision that the public should be involved in the Section 4 data review process to the extent possible. EPA will strive to assure that non-confidential information be available to the public as soon as possible.

Finally, the substantiation burden is further justified by the need to discourage ill-founded claims. Substantiation aids the submitter in understanding the findings which must be made to support a confidentiality claim. Experience with the inventory reporting regulations indicates that detailed substantiation requirements significantly reduced the number of claims. This should result in a higher level of public information, and a reduced burden on EPA to evaluate and protect information erroneously claimed to be confidential.

EPA considered requiring substantiation of a claim of confidentiality for information contained in health and safety studies to be submitted only after receipt of an FOIA request for the information.

However, as indicated above, EPA believes that this policy would not accomplish a number of purposes EPA believes necessary to an effective implementation of TSCA:

- speedy response to FOIA requests
- lessened administrative burden on the Agency
- discouragement of overly broad confidentiality claims

EPA's proposed Section 4 policy concerning the substantiation of confidentiality claims for information contained in health and safety studies is similar to those proposed under the Section 5 Premanufacture notification rulemaking (44 FR 2242, January 10, 1979).

B. Substantiation Questions

EPA's proposed procedures for Section 4 would require persons claiming confidentiality or health and safety data to address the following questions:

1. Will disclosure of the information claimed as confidential in your health and safety study disclosure a process used in manufacturing or processing a chemical substance or mixture? If so, describe how the information will disclose the process.

2. Will disclosure of the information claimed as confidential in your health and safety study disclose proportions of a mixture comprising any of the chemical substances in the mixture? If so, describe how the information will disclose the proportions.

3. Will disclosure of the information claimed as confidential in your health and safety study disclose information which is not related in any way to the effects of a substance on health or the environment? If so, describe how the information will disclose this information.

4. Has any of the information claimed as confidential been disclosed on a patent? If so, why should it be treated as confidential?

5. Is this information available to the public or your competitors without restriction?

6. How do you protect this information from undesired disclosure?

7. Has this information been disclosed to others? If so, what precautions have you taken in regard to these disclosures? Has the information been disclosed to the public or competitors?

8. In the case of information concerning proportions of a mixture comprising one or more chemical substances, does the mixture leave the control of your company and move in commerce? If so, can the mixture be analyzed to determine the proportion of the chemical substance in it?

9. Has EPA, another Federal Agency, or any Federal court made any pertinent confidentiality determinations regarding this information? If so, please attach copies of such determinations.

10. How long should confidential treatment be given? Until a specific date, the occurrence of a specific event, or permanently? Why?

11. What harmful effects to your competitive position, if any, do you think would result from disclosure of the process? The proportions? Information not related in any way to the effects of a substance on the health or the environment? How would a competitor use this information? Would the harmful effects be substantial? What is the causal relationship between disclosure and the harmful effects?

V. Differences Between TSCA Section 4(b) Test Standards and FIFRA Guidelines

A. Introduction

EPA's Office of Pesticide Programs has proposed testing guidelines (43 FR 37336, Aug. 22, 1978) under the authority of Sections 3(c)(2) and 25(a) of the Federal Insecticide, Fungicide, and Rodenticide Act as amended (FIFRA) (86 stat. 973; 89 stat. 751; 7 U.S.C. 136 et seq.), which contain data requirements comparable to those presently being proposed under TSCA Section 4(b). The Agency's policy is to reduce the burden on the regulated public which might arise from conflicting requirements under these different sets of regulations Therefore, although the proposed TSCA test standards and FIFRA guidelines differ in some significant ways, the final TSCA test standards and FIFRA guidelines will be consistent to the extent permitted by the different laws. The major differences between the two proposed regulations are identified below to help facilitate the comparison of the two proposals by the public.

B. Section 772.113-1 General

1. Subsection(d): The FIFRA guidelines (Section 163.80-3(b) (5-7,12) do not contain a separate section on good laboratory practice standards as do the TSCA test standards. Good laboratory practice standards appear throughout the FIFRA guidelines and are less detailed in some respects.

2. Subsection(e): The FIFRA guidelines (Section 163.80-3(b)(1)) do not contain as detailed personnel requirements as the TSCA test standards do. For example, the TSCA test standards have proposed two types of qualified pathologists with specified training and experience and do not presently allow for substitution of persons with equivalent training and experience as the FIFRA guidelines do.

3. Subsection(f): The TSCA test standards require the submission of a study plan at least 90 days before a study is initiated. The FIFRA guidelines do not require submission of a study plan prior to study initiation but do require submission of a test protocol with the final report (Section 163.83-1). A study plan submission requires other information in addition to the test protocol.

4. Subsection(g): The TSCA test standards specify that the test substance or mixture administered must contain no less than 90 percent of the specified test substance concentration during the time of

administration; FIFRA guidelines specify only that no mixture of test or control substance be maintained or used during the period exceeding the known stability of the test or control substance. Also, only the TSCA standards specified that the initial mean concentration of the test substance must not vary more than 5% from the concentration designated in the test protocol.

5. Subsection(h-i): Only the TSCA test standards require that all feed be used within 90 days of its manufacture, that all rodents be fed a specified standardized diet, and that feed and vehicles be analyzed for specified contaminants.

6. Subsection(j): The TSCA test standards require the submission of "Interim Quarterly Summary Reports." FIFRA guidelines do not have such a requirement.

C. Section 772.113-2 Oncogenic Effects Test Standards

1. Subsection(a)(3): The TSCA test standards require that animals be weaned and environmentally acclimatized before dosing; the FIFRA guidelines (Section 163.83-2(c)(3)), under certain specified circumstances, permit dosing *in utero*.

2. Subsection(a)(5): The FIFRA guidelines (Section 163.83-2(c)(4)) require both an untreated and a vehicle control group, if the toxic properties of the vehicle are unknown. The TSCA test standards leave the decision to the discretion of the tester. The TSCA test standards indicate positive control groups may be required for particular chemicals.

3. Subsection(a)(7): The TSCA test standards specify the frequency of exposure for the different routes of exposure; the FIFRA guidelines do not.

4. Subsection(a)(8): The TSCA test standards require the tester to administer the test substance to both rats and mice for a minimum of 24 months but no longer than 30 months. The FIFRA guidelines (Section 163.83-2(c)(6)) require the test substance to be administered to mice for a minimum of 18 months and not ordinarily longer than 24 months; to rats 24 months and 30 months, respectively.

5. Subsection(a)(9): Both the TSCA test standards and FIFRA guidelines (Section 163.83-2(c)(7)) require at least three dose levels in addition to controls. However, they define the highest dose level and lowest dose level slightly differently. The TSCA test standards require a preliminary toxicology study of at least 90 days to select the dose levels and require the sponsor to submit the rationale for dose selection as part of the study plan submission. FIFRA guidelines state that dose levels are generally predicted from subchronic data.

6. Subsection(b)(1): The TSCA test standards require that qualified veterinarian(s) be responsible for the health status and care of all test animals. The FIFRA guidelines do not specify the qualifications of the veterinarian.

7. Subsection(b)(1): The TSCA standards provide that the animals must be observed every 12 hours and that losses greater than 5% in any group due to cannibalism, autolysis of tissues, misplacement of animals, and similar management problems

may not be acceptable. The FIFRA guidelines (Section 163.83-2(c)(10)) require each test animal be observed at least daily, with losses not to exceed 10 percent.

8. Subsection(b)(2): The TSCA test standards specify the responsibilities of the two types of qualified pathologists.

9. Subsection(b)(2): The tissues to be examined microscopically are essentially the same for both regulations. However, only the TSCA test standards require oral mucous membranes and aorta be examined and only the FIFRA guidelines require routine examination of the sciatic nerve.

D. Section 772.113-3 Non-oncogenic Chronic Effects Test Standards

1. Subsection (a)(1): The TSCA test standards require in addition to a rodent, generally the rat, the use of a non-rodent, generally the dog.

2. Subsections 772.113-2(a)(3,5,7): The differences in these subsections have been discussed above in part C, paragraphs 1-3.

3. Subsection 772.113-3(a)(8): The TSCA test standards require the tester to administer the test substance to the rat for at least 30 months. The FIFRA guidelines (Section 163.83-1(c)(6)) require administration for at least 24 months but no longer than 30 months. In addition, the TSCA test standards require the test substance be administered to dogs for at least 2 years.

4. Subsection 772-113-3(a)(9): FIFRA guidelines (Section 163.83-1(c)(7)) require that the highest dose be higher than that expected for human exposure. Under the TSCA test standards the tester must conduct a preliminary toxicology study of at least 90 days to select the dose levels and must submit the rationale for dose selection as a part of the study plan submission.

5. Subsection 772-113-3(b)(1)(i): The differences in this subsection have been discussed above in part C, paragraphs 6-8.

6. Subsection(b)(1)(i): The TSCA test standards require that urinalysis and certain specified function test be performed while the FIFRA guidelines leave these decisions to the discretion of the tester.

7. Subsection 772-113-3(b)(2)(ii): The TSCA test standards require that all of the tissues listed in this subsection be examined from all test animals. The FIFRA guidelines (Section 163.83-1(c)(16)) require a limited number of tissues from all test animals be examined and other specified tissues from test animals in certain test groups be examined.

E. Section 772.113-4 Combined Chronic Effects Test Standards

FIFRA guidelines (Section 163.80-5) allow combined testing to be conducted if the data requirements of each individual test are satisfied. They do not, however, provide any specified test methods. TSCA Section 4 test standards (Section 772.113-4) propose a test method for studying both oncogenic and chronic toxicity effects simultaneously.

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